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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1979

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TECHNICAL REVIEW AND APPROVAL

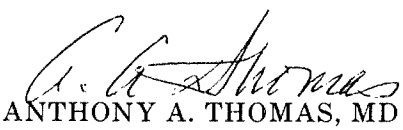
AMRL-TR-79-56

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



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1,1-Dimethylhydrazine	Fluomine	JP-10
1,2-Dimethylhydrazine	Jet Fuels	DFM
Hydrazine	JP-5	Diesel Fuel
Monomethylhydrazine	JP-9	OMP-1 (Cont'd)

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1978 through May 1979 are reviewed in this report. Chronic toxicity or oncogenic studies were carried out with inhaled methylcyclohexane, tricyclodecane, and purified 1,1-dimethylhydrazine. A subchronic inhalation study was conducted with decalin. Acute oral and intraperitoneal toxicity studies were conducted on a variety of chemical agents used by the Air Force and Navy. Sensitization, eye, and skin irritation studies were also made on these chemicals.

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BLOCK 19.

OMP-2
Antifouling Paints
Methylcyclohexane
Organometallic
Toxicity
Carcinogenesis
Oncogenesis
Emergency Exposure Limits
Sensitization
Oral
Intraperitoneal
Irritation
Skin
Eye
Acute
Chronic
Inhalation
Tricyclodecane
Decalin
Tetrahydronaphthalene
Shale Oil

PREFACE

This is the sixteenth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-76-C-5005. This document constitutes the fourth report under the current contract and describes the accomplishments of the THRU from June 1978 through May 1979.

The current contract for operation of the Laboratory was initiated in 1975 under Project 6302 "Occupational and Environmental Toxic Hazards in Air Force Operations," Task 01 "Toxicology of Propellants and Materials," Work Unit Number 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to A. K. Roy-Chowdhury, Ph.D., C. E. Johnson, C. C. Haun, and G. L. Fogle for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the U.S. Naval Medical Research Institute and the Department of Transportation.

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SECTION I

INTRODUCTION

This document constitutes the 16th annual report of the Toxic Hazards Research Unit (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories which have previously been described by MacEwen (1965), Fairchild (1967), and Thomas (1968).

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems, and the potential oncogenicity of chemicals used in military and civilian activities. To this end, many of the current research programs serve the mutual interests of the U. S. Air Force, U. S. Navy, and other governmental agencies.

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to the U. S. Air Force and other governmental and industrial scientists. This year's conference, chaired by Colonel Vernon L. Carter, Jr., USAF, VC, presented 27 technical papers covering a broad range of occupational and environmental toxicology problems. Seven papers were presented by University of California faculty and staff members. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 28 March through 30 March 1979, drew 164 participants including speakers.

The papers presented at the conference were prepared for publication as the Proceedings of the 9th Conference on Environmental Toxicology which will be a separate technical report.

Our next conference, currently in the development stage, will be held in November 1979 at the Imperial House South Motel, Dayton, Ohio. The difficulty in scheduling a conference without major conflict with other important environmental health meetings in the spring of the year has prompted the return to holding the conference in the fall. November was selected to avoid travel funding problems for many government workers who attend this conference.

SECTION II

RESEARCH PROGRAM

The research activity of the THRU is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broad range of chemical materials and includes inhalation studies of rocket and aircraft fuels. Acute oral and dermal toxicity studies on a variety of materials were also conducted.

A STUDY OF THE ONCOGENIC CAPACITY OF INHALED MONOMETHYLHYDRAZINE

Hydrazines administered in the drinking water of Swiss mice and Golden Syrian hamsters have been reported by Toth (1972, 1973) to have carcinogenic activity. In the first of these studies, solutions of 0.001% methylhydrazine sulfate were given daily ad libitum to 5 and 6 week old randomly bred Swiss mice for their entire lifetimes. Hydrazine and methylhydrazine sulfate significantly increased the incidence of lung tumors in the mice, while methylhydrazine enhanced the development of neoplasms by shortening the latent period. In the second of Toth's studies, Golden Syrian hamsters received 0.01% methylhydrazine in drinking water daily ad libitum for life. Malignant histocytomas (Kupffer cell sarcomas) were observed in the livers of 54% of the male hamsters treated, while none were observed in the control groups.

Earlier studies of MMH carcinogenicity by Kelly et al. (1969) and Roe et al. (1967) did not demonstrate any increase in tumor incidence over control animals. Roe administered 0.5 mg MMH per day by mouth to Swiss mice on a 5 day/week for 40 weeks schedule and found a lower incidence rate of tumor bearing mice (pulmonary adenomas) compared to untreated controls. The one exposed mouse that had tumors had many more adenomas than the control mice of the tumor bearing controls. Kelly reported *per os* administration of 0.2 ml MMH solution/mouse to female CDF_1 mice and i.p. administration of 0.1 ml MMH solution/mouse in male mice of the same strain produced no more lung adenomas or leukemias than were found in untreated controls after 8 weeks of treatment. The MMH was given in a 2% aqueous solution.

MacEwen and Vernot (1975) reported the results of a two year drinking water study in which hamsters were given standard and acidified drinking water containing 0.01% MMH. A third group of hamsters was given acidified water (pH 3.0) as unexposed controls.

Neither the incidence, degree of severity, nor age of onset of nonneoplastic pathologic changes was markedly different between animals drinking MMH in water and control animals. The presence of 23% incidence of adrenocortical tumors in control animals versus 4% in Group I (MMH in tap water) and 12% in Group II (MMH + pH 3.5 water) argues against MMH as a cause of these tumors. The remaining neoplasms, one hemangioendothelioma of the liver, two hepatocellular carcinomas, one cutaneous melanoma, occurred only in the experimental groups. They were derived from four different cell types and as such constitute a 4% incidence for each tumor in their respective groups of animals, except for an 8% incidence of hepatocellular carcinoma. The overall tumor incidence for Group I (MMH + tap water) was 16%, Group II (MMH + pH 3.5 water) was 24%, and Group III (control) was 31%. These findings are in contrast to the findings of Toth and Shimizu (1973).

The reported investigations present some evidence that MMH may be carcinogenic and therefore may pose a hazard to man. The case for carcinogenicity of MMH is, however, inconclusive at this point and for this reason, the comprehensive inhalation exposure study described herein was undertaken.

Rats, mice, hamsters, and dogs were exposed to MMH by the inhalation route in chambers for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off to simulate a human exposure regimen.

All rodents were held for an additional year of observation at which time necropsies were performed on survivors and approximately 33 tissues were taken for histopathologic evaluation of tumorigenesis following the National Cancer Institute protocol. The dogs will continue to be held for additional postexposure observation. They are located at the vivarium at Wright-Patterson Air Force Base, Ohio.

The previous annual report (MacEwen and Vernot, 1978) contains experimental data including mortality, body weight measurements, and clinical chemistry results of dogs tested during the 12 months of MMH exposure and through two months postexposure.

At the present time, all mice and rats are dead. They either died or were sacrificed due to moribund condition during the postexposure phase of the study or were sacrificed following the 12-month postexposure observation period. The hamsters are scheduled for necropsy in June 1979, and the dogs will continue to be held and examined until March 1984. The dogs receive semiannual comprehensive physical examinations and clinical chemistry determinations.

The hamsters had not concluded the exposure portion of the experiment at the time of the last report. The following table (Table 1) shows the mortality experience in all rodents after 52 weeks of exposure.

TABLE 1. MORTALITY EXPERIENCE IN RODENTS DURING A 12-MONTH INHALATION EXPOSURE TO MONOMETHYLHYDRAZINE (MMH)

Species, Sex	Exposure Time, Weeks	Unexposed Controls	Exposure Concentration 0.02 ppm	0.2 ppm	2.0 ppm	5.0 ppm
Mice, ♂	52	44/400	63/400	51/400	39/400	--
Rats, ♂	52	6/150	1/100	3/100	10/100	10/100
Rats, ♀	52	4/150	2/100	4/100	7/100	20/100
Hamsters, ♂	52	23/150	--	33/200	44/200	64/200

A dose-response relationship in mortality rate is not present in the mice or the male rats. The female rats show a higher mortality incidence at the 5 ppm level which does appear to be related to concentration, and the hamsters show an increase in mortality paralleling the increase in concentration at each exposure level.

By the conclusion of the one-year postexposure observation period, all dose-response in mortality has disappeared as shown in Table 2.

TABLE 2. CUMULATIVE MORTALITY IN MMH EXPOSED RODENTS

Species, Sex	Time in Study, (Months)	Unexposed Controls	Exposure Concentration 0.02 ppm	0.2 ppm	2.0 ppm	5.0 ppm
Male, ♂	24	200/400	216/400	191/400	199/400	--
Rats, ♂	24	48/150	22/100	16/100	11/100	24/100
Rats, ♀	24	52/150	26/100	28/100	25/100	34/100
Hamsters, ♂	23	144/200	--	113/200	122/200	121/200

The growth of male rats showed a dose response to MMH exposure throughout the entire study. This was particularly evident at the 5 and 2 ppm concentrations (Figure 1) where a large depression in mean growth is evident. The effects were less noticeable at the 0.2 and 0.02 ppm MMH exposure levels but were statistically different from the unexposed controls throughout the entire exposure period. All exposure levels continued to show a statistically significant depression in mean body weights when compared to their respective control group for the entire 52 weeks postexposure observation period. The mean body weights of the female rats (Figure 2) were more sporadic in nature; however, the two highest exposure concentration groups remained significantly below the control group for the duration of the exposure period. With the exception of the 2 ppm group at 16 weeks postexposure(68 weeks from study initiation), all MMH exposed female rat groups were statistically lower in mean body weight from 12 weeks postexposure through 52 weeks postexposure.

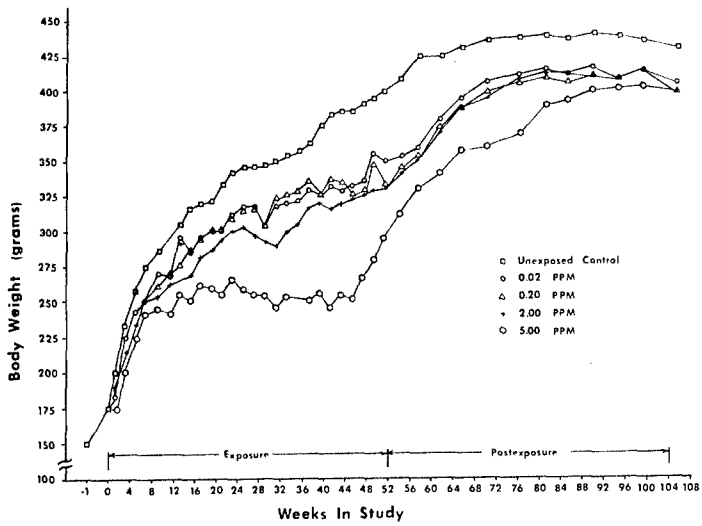


Figure 1. The effect of chronic inhalation exposure to MMH on the growth rate of male rats.

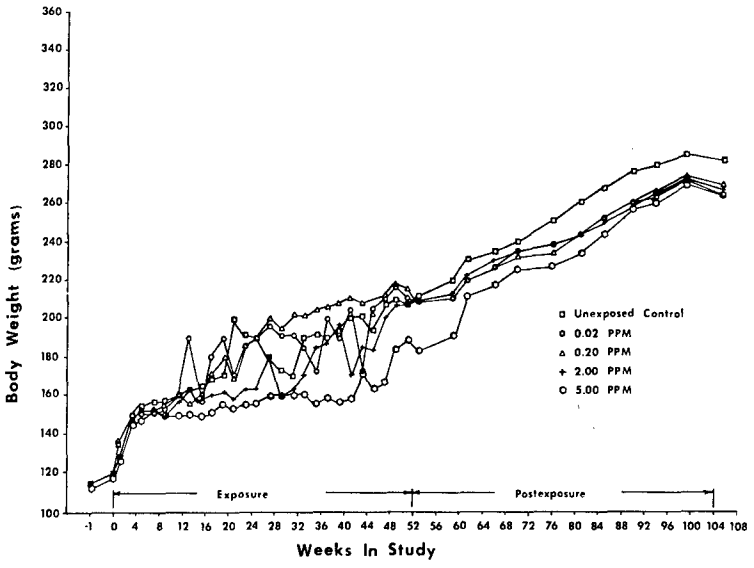


Figure 2. The effect of chronic inhalation exposure to MMH on the growth rate of female rats.

The mean weight of the 5 ppm MMH exposure group of hamsters (Figure 3) showed a definite depression when compared to the unexposed control group. The two intermediate concentration levels remained below control values in most cases, but did not show a clear dose response as was seen in the male rats. After the first week of exposure, the 5 ppm and 2 ppm exposed groups were statistically different from the controls. After 19 weeks of exposure, all of the test groups were statistically lighter than the controls. Unlike the rats, the MMH exposed hamsters were able to recover this difference in weight during the postexposure phase of the study.

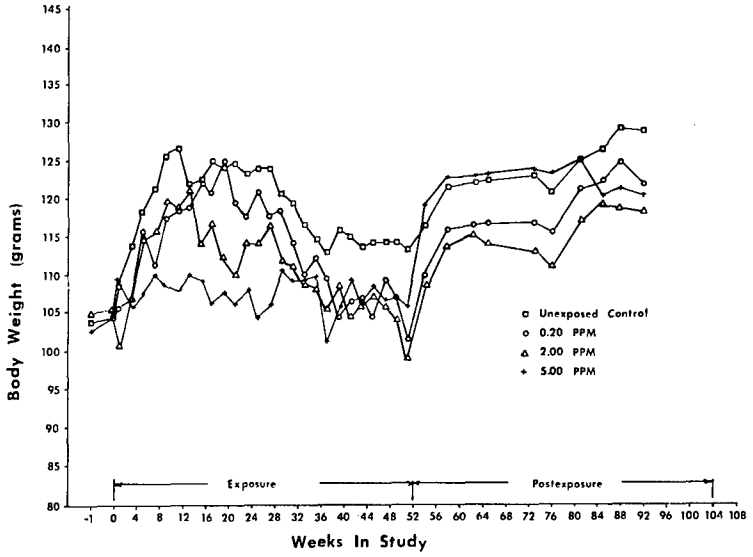


Figure 3. The effect of chronic inhalation exposure to MMH on the growth rate of male Golden Syrian hamsters.

The growth of MMH exposed beagle dogs is comparable to their controls one year postexposure and all blood parameters have continued within normal ranges during the same period.

The results of histopathologic examination are not yet available and may be reported on in the next annual report.

A 6-MONTH CHRONIC INHALATION EXPOSURE OF ANIMALS TO UDMH TO DETERMINE ITS ONCOGENIC POTENTIAL

Preliminary evidence that hydrazine (N_2H_4) was carcinogenic at concentrations near or at the industrial TLV (MacEwen and Vernot, 1974) led to concern for the oncogenic potential of unsymmetrical dimethylhydrazine (UDMH), another important military chemical which had been reported to be tumorigenic in animals by Roe et al. (1967), Toth (1972, 1973), and Druckrey et al. (1967). This concern resulted in a series of chronic inhalation toxicity experiments conducted to examine the hazard associated with UDMH exposure.

Four animal species were used in this study. The concentration levels used at or near the current TLV of 0.5 ppm were 5, 0.5 and 0.05 ppm. Exposures conducted were of the industrial type, 6 hours/day, 5 days/week for six months. This was followed by a prolonged observation period for potential tumor induction in each species. Animals and the number used in each exposed and control group were 400 female C57Bl/6 mice, 200 male Fischer 344 rats, 200 male Golden Syrian hamsters, 4 male and 4 female beagles. A concurrent set of control animals was provided for the 0.05 ppm test as it was not started at the same time as the 5 and 0.5 ppm experiments. Dogs and rats were housed in one dome and mice and hamsters in a companion chamber. All control animals were maintained in animal housing facilities.

The inhalation exposure chambers were operated with nominal air flows of 35 CFM at a slightly reduced pressure of 725 mm Hg to prevent leakage of UDMH into the laboratory. Nearly continuous monitoring of chamber concentrations was performed with an AutoAnalzyer, the same instrumentation and techniques used for chronic hydrazine and MMH studies (Haun, 1970; MacEwen and Haun, 1971; Haun and Kinkead, 1973). The commercial UDMH used in these studies contained 0.12% dimethylnitrosamine (DMNA) which is a known potent carcinogen. The primary commercial method of manufacture of UDMH uses DMNA as the starting material, and the finished product usually contained some residue of the DMNA. This may be a significant consideration in the assessment of oncogenic effects of UDMH inhalation.

All animals were observed hourly during exposure and nonexposure periods for signs of UDMH intoxication and mortality. Gross and histopathologic examinations were done on all dead animals. Rats, hamsters and dogs were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups and group mean weights followed on a monthly basis throughout the experimental period.

Blood samples were drawn from dogs at biweekly intervals, and clinical determinations were done for the battery of tests shown in Table 3.

TABLE 3. CLINICAL BLOOD TESTS PERFORMED ON DOGS EXPOSED TO 5, 0.5, AND 0.05 PPM UDMH AND CONTROLS

RBC	Sodium	Albumin
WBC	Potassium	Globulin
HCT	Calcium	SGPT
HGB	Glucose	Alkaline Phosphatase
Differential Cell Count	Total Protein	

Blood measurements not included in the regular biweekly schedule during the exposure phase of the study but done at conclusion of the 5 ppm and 0.5 ppm exposures are shown in Table 4. Of these, tests giving abnormal values were repeated postexposure at regular intervals until recovery. To examine for possible hemolytic effects in rodents, blood samples for hematocrit and red blood cell counts were taken from 5 rats and 5 hamsters from each group at the conclusion of the 5 ppm and 0.5 ppm exposures.

TABLE 4. SUPPLEMENTAL CLINICAL BLOOD TESTS PERFORMED ON DOGS AT THE END OF EXPOSURE TO 5 AND 0.5 UDMH AND CONTROLS

Blood Urea Nitrogen	SGOT
Chloride	Prothrombin Time
Cholesterol	Cephalin Flocculation
Creatinine	Bromsulphalein Retention

Significant exposure effects of UDMH were limited to slight to moderate, but transitory, hepatotoxicity in dogs exposed to the 5 ppm concentration, as shown in Table 5. Dogs exposed to the 5 ppm level developed significantly elevated serum glutamic pyruvic transaminase (SGPT) levels by the fourth week of exposure. At 6 weeks, the mean SGPT value for the exposed dogs was 3 times the control level. Throughout the remaining 20 weeks of exposure, values for the exposed dogs were stable at levels 3 to 4 times those of the control group.

TABLE 5. EFFECT OF 6-MONTH INHALATION EXPOSURE TO 5 PPM UDMH ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS IN DOGS

[Group Mean Values (N=8)]

Weeks of Exposure	Control Group	5 ppm Group
2	26[1]	32
4	27	79*
6	27	102*
8	25	118*
10	26	118*
12	31	116*
14	--	---
16	22	88*
18	23	107*
20	23	99*
22	20	97*
24	22	100*
26	25	86*

[1]International Units.
*Significant at the 0.01 level.

At shown in Table 6, there is a trend to recovery, approximately a 50% reduction, in measurements done at 2 weeks postexposure. Subsequent values at 4, 8, and 11 weeks postexposure showed no further reductions. However, when the dogs were sampled again (at Brooks AFB where they were being maintained) at 27 and 47 weeks postexposure, values were completely normal when compared with control animal values. Periodic measurements have been made since that time and are continuing. All values have fallen to within normal ranges.

TABLE 6. POSTEXPOSURE EFFECT OF 6-MONTH INHALATION EXPOSURE TO 5 PPM UDMH ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS IN DOGS

[Group Mean Values (N=8)]

Weeks Postexposure	Control Group	5 ppm Group
2	22[1]	37*
4	23	42*
8	22	36*
11	23	35*
27**	33	30
47**	40	37

[1]International Units.
*Significant at the 0.01 level.
**Measurements made at Brooks AFB.

Liver function tests were performed on dogs at exposure termination and at 4, 8, 11, and 38 weeks postexposure. This information is shown in Table 7. Bromsulphalein (BSP) measured in the blood of the 5 ppm UDMH exposed dogs 10 minutes following a 10 mg/kg injection showed significant retention at exposure termination, 4 and 8 weeks postexposure. Recovery occurred at 11 weeks postexposure. BSP measurements done at Brooks Air Force Base 38 weeks postexposure show no abnormal values for the exposed dogs. Subsequent scheduled measurements have also been normal.

TABLE 7. MEAN BROMSULPHALEIN RETENTION VALUES* IN CONTROL AND 5 PPM UDMH EXPOSED DOGS

Time	Control	5 ppm
Exposure Termination		
26 Weeks	18.1	30.3**
Weeks Postexposure		
4 Weeks	20.7	29.5**
8 Weeks	12.8	30.0**
11 Weeks	18.0	21.8
38[1] Weeks	11.4	12.3

*Percent retention.
**Significantly higher than controls at the 0.05 level.
[1]Measurements made at Brooks Air Force Base.

Examination of hematocrit and RBC determinations done immediately postexposure on rats and hamsters showed no abnormalities. Likewise, clinical results on dogs other than those mentioned showed no effects of exposure to UDMH.

Mean body weights for all groups of exposed rats and hamsters were depressed during the exposure phase of the study, but they were not dose related. The weights of dogs and mice were unaffected by UDMH exposure.

Mortality, during exposure, was limited. No dogs died, a few rats and mice were found dead, but in no case was death attributed to UDMH exposure. A malfunctioning water valve with hamsters early in the 5 and 0.5 ppm experiments caused a number of accidental deaths by drowning in these test groups. The problem was successfully corrected.

The protocol for microscopic examination of tissue was that used by the National Cancer Institute biocollaborative carcinogenesis research program. The nontumor pathology results have not been completely analyzed. However, there were no striking changes in exposed animals when compared with their controls.

One male dog in the high dose group died one year and 9 months following the start of the study. The cause of death and the only tumor seen in this dog was a metastatic reticulum cell sarcoma that most likely arose in the mediastinum. A dog in the low dose group was euthanized 4 years and 2 months following the start of the study because of posterior paresia. The cause of paralysis was a ruptured intervertebral disc. No neoplasms were seen in this dog. All other dogs are in good health.

Microscopic examination of hamster tissues was done by a group of USAF veterinary pathologists at Brooks AFB. Tumor nomenclature was developed for an automated data processing capability to compile results from hamsters. Incidence tables and statistical analysis of results was done by the THRU. There were no tumor types seen in these hamsters that were significantly increased over those seen in controls. The incidence of tumors seen in these hamsters is presented in Table 8.

The tumor incidence seen in rat and mouse tissues was tabulated in the previous annual report (MacEwen and Vernot, 1978).

TABLE 8. TUMOR INCIDENCE IN CONTROL AND UDMH EXPOSED GOLDEN SYRIAN HAMSTERS

Tumor Type	Unexposed Control	0.05 ppm Exposed	0.5 ppm Exposed	5.0 ppm Exposed	Unexposed Control
Lung					
Pulmonary Sarcoma	0/190	0/178	3/166	1/158	0/167
Pleural Mesothelioma	1/190	1/178	0/166	0/158	0/167
Bronchogenic Adenoma	1/190	0/178	0/166	0/158	0/167
Tracheobronchial Adenoma	1/190	0/178	0/166	0/158	0/167
Adrenal					
Cortical Adenoma	29/185	33/167	27/146	25/138	25/149
Medulllary Adenoma	2/185	2/167	7/146	1/138	3/149
Bone Marrow Tumors	2/168	1/148	0/128	3/121	2/129
Reticulo-Endothelial System					
Reticulo-Endotheliomas	20/171	13/144	16/171	10/164	9/177
Gastro-Intestinal System					
Esophageal Papilloma	0/179	0/172	1/197	0/140	0/154
Stomach Papilloma	1/182	1/169	1/148	0/148	0/160
Stomach Adenocarcinoma	0/182	1/169	0/148	1/148	0/160
Colon Papilloma	1/149	0/143	0/84	0/99	0/82
Colon Adenocarcinoma	1/149	0/143	0/84	0/99	0/82
Thyroid					
Thyroid Adenoma	0/147	1/131	0/83	0/75	1/85
"C" Cell Adenoma	0/147	3/131	0/83	2/75	0/85
Parathyroid Adenoma	1/87	0/73	1/28	1/27	1/29
Skin					
Trichoepithelioma	0/193	0/184	1/171	0/164	0/177
Melanoma	1/193	0/184	0/171	0/164	0/177
Undifferentiated Tumor, Malignant	1/193	0/184	0/171	0/164	0/177
Kidney					
Adenoma	1/191	0/177	0/168	0/155	2/170
Other Tissues					
Salivary Gland Adenoma	0/193	0/184	0/171	0/164	1/177
Gingiva Epithelioma	0/193	0/184	1/171	0/164	0/177

The outstanding finding in mice was the increase in hemangiosarcomas and Kupffer cell sarcomas. In the 5 ppm UDMH exposure group, there were 19 hemangiosarcomas versus 3 in unexposed controls. Also, 8 Kupffer cell sarcomas are seen compared with none in the control group. The numbers are modest, but significant in the 5 ppm group. There is a

fairly large incidence of malignant lymphomas in each group of exposed and control mice, but it is important to note that incidence in the 5 ppm group is statistically higher than the incidence in the controls.

Two types of lung tumors were observed in rats. Bronchiolar adenomas are somewhat more prevalent. There was a statistically significant increase of these tumors in the high UDMH dose group. Although there is no statistical validity, squamous cell carcinomas are conspicuous in that 4 such tumors were found in the high dose group but none in any of the other control or exposed groups. The incidence of these tumors occurring spontaneously in rats is quite low. Hepatocellular carcinomas were seen in six rats in the 5 ppm exposed group. This tumor type was not seen in the controls. A significant increase in islet cell adenomas is seen in the 0.5 ppm dose group. The high dose group does show a higher incidence than its controls, but this is not statistically significant. Chromophobe adenomas are a highly prevalent tumor in the aged Fischer 344 rats. Both the high and the intermediate dose groups show a significantly higher incidence than controls. Brain tumors of the central nervous system are rare in rats, but seven were seen in exposed animals, none in controls. There was no statistically significant increase in any one type seen. Four of the seven tumors were astrocytomas, which is in keeping with reports that astrocytomas are the most prevalent type of brain tumor seen in rats. Fibrous histiocytomas are a fairly common tumor seen in the skin and soft tissue of rats. There were increases of this type seen in both the high and intermediate dose groups, statistically significant at the high dose level only.

In summary of tumor incidence, a dose related tumor response was not seen in the data for mice. It was seen in most of the tumor types for rats, but without statistical significance.

Overall total tumor incidence was greater in all exposed rats and mice than in their controls. Statistical evidence for significant tumor production was seen in rats and mice exposed to the highest dose used. Of particular importance are the hepatocellular carcinomas in rats and the hemogiosarcomas and Kupffer cell sarcomas in mice which we believe to have been caused by the DMNA impurity in the MMH.

A STUDY OF THE ONCOGENIC POTENTIAL OF PURIFIED UDMH IN MICE

Unsymmetrical 1,1-dimethylhydrazine (UDMH) is a missile fuel used by the military which has been reported to be carcinogenic in animals (Roe, 1967; Toth, 1972 and 1973).

Toth (1973) describes a lifetime exposure of 5-week old Swiss mice to a 0.01% solution of UDMH in the drinking water. The treatment in this study significantly shortened the survival time of the treated animals compared to untreated controls. Tumors of the blood vessels (79%), lungs (71%), kidneys (10%), and livers (6%) were found with the majority (78%) of the vascular tumors being in the liver.

The suggestion that UDMH is carcinogenic has lead to studies involving the inhalation of UDMH vapors. A recent study by MacEwen and Vernot (1976) involved exposure of 4 animal species to 5, 0.5 or 0.05 ppm UDMH for a period of 6 months on an intermittent (5 days/week, 6 hours/day) schedule. The major nononcogenic finding in this study was a slight hepatotoxic effect, as characterized by increased SGPT levels and increased BSP retention time, in the dogs exposed to the 5 ppm concentration. These observations had not previously been reported by other investigators studying UDMH. Histologic examination of the tissues of rats and mice exposed to UDMH revealed an increased incidence of tumors, particularly those occurring in the liver.

The UDMH used in the oncogenic studies discussed earlier in this report was manufactured by a process that used n-nitrosodimethylamine (DMNA) as a starting material and 0.12% of this material remained in the finished product as a common contaminant. Dimethylnitrosamine is a known liver toxin and carcinogen (Magee and Barnes, 1956, 1967; Terracini et al., 1967). Haun (1976) speculated that the contaminating DMNA was the agent responsible for the hepatotoxic effects observed in the 6-month inhalation study. He exposed dogs to purified UDMH for $8\frac{1}{2}$ weeks and found no increase in SGPT levels. A second group of dogs exposed for 16 days to UDMH, spiked to contain 0.12% DMNA, did show significantly elevated SGPT levels, but there was no increase in BSP retention time nor was there any visible cellular damage in the liver.

The incidence of transitory hepatotoxicity observed in dogs exposed to 5 ppm UDMH for 6 months appears to be linked to the DMNA which contaminated the test sample. The incidence of blood vessel tumors in the livers of mice exposed to UDMH cannot be conclusively tied to UDMH since there was simultaneous exposure to DMNA. DMNA is a potent liver toxin which

raises a question as to which material may be responsible for the incidence of liver hemangiosarcomas. The objective of this study was to determine the oncogenic potential of purified UDMH (DMNA removed by distillation) in mice.

MATERIALS AND METHODS

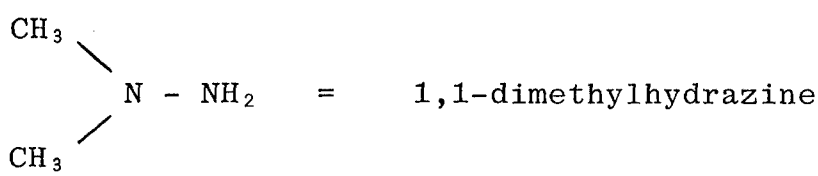
The unsymmetrical dimethylhydrazine used in this experiment was redistilled from propellant grade UDMH to remove most of the dimethylnitrosamine. The redistilled UDMH was stored in teflon-lined, capped, brown 100 ml glass bottles under nitrogen. This storage method has been shown to prevent oxidation degradation of monomethylhydrazine for periods greater than one year.

Each bottle of UDMH was analyzed for DMNA content before and during use using a gas chromatographic method developed in our laboratory. The DMNA concentration in the UDMH used in the study was less than the lower detection limit of 5 $\mu g/\ell$.

A Sage Model 355 syringe with a 10 ml glass syringe set up in a contaminant introduction hood is used for chamber UDMH introduction, one for each chamber. The UDMH is evaporated in a 500 ml/minute sample air stream without additional heat. The evaporated sample is then introduced into the 20 cfm chamber air stream. Reaction of UDMH with chamber ducts and walls results in a loss of about 10% at equilibrium.

The chamber UDMH concentrations are sampled from a spot just above the mice breathing zone. The sample is pulled through polyethylene tubes to an electric two-way valve which samples for 10 minutes from one chamber and then 10 minutes from the other. The samples are analyzed with an MDA Model 7020 hydrazine analyzer using a sample flow of 31 cc/minute. The switch and analyzer are mounted between the chambers so as to reduce the distance of sample travel. This is necessary with low sample flow to reduce the switching equilibrium time. Each MDA tape is calibrated before use and one calibration point run each week thereafter.

The structure and physical properties of UDMH are shown below.



= 1,1-dimethylhydrazine

M. W.	- 60.1
Liquid Density	- 0.784 g/cc at 25 C
Boiling Point	- 63 C
Critical Temperature	- 250 C
Flash Point (Closed Cup)	- 1.1 C
Vapor Pressure	- 15.5 mm Hg at -17.8 C
	51.8 mm Hg at 4.4 C
	160.5 mm Hg at 26.7 C
	435.0 mm Hg at 48.9 C

Female mice (C57Bl/6) are being exposed for one year to 5.0 ppm purified UDMH in Rochester chambers of 2 m^3 volume. The exposures are conducted following an industrial work week type schedule of 5 days/week, 6 hours/day with no exposures on weekends or holidays. These exposures simulate human exposure situations and parallel the conditions of the previous 6-month UDMH exposure. An equal number of mice serving as controls are maintained in Longley chambers 2.5 m^3 in volume.

At the conclusion of the year long exposure, all animals will be held for postexposure observation. This observation period will last for one year or until the cumulative mortality of the group exceeds 90%. At either of these times, surviving animals will be sacrificed, necropsies performed and tissues listed in Table 9 taken for histologic evaluation of tumorigenesis.

TABLE 9. TISSUES SAMPLED FROM MICE EXPOSED TO PURIFIED UDMH VAPORS

Gross lesions	Anus
Tissues masses or suspect tumors and regional lymph nodes	Mesenteric lymph node
	Liver
	Thigh muscle
Skin	Sciatic nerve
Mandibular lymph node	Sternebrae, vertebrae, or femur (plus marrow)
Mammary gland	
Salivary gland	Thymus
Larynx	Gall bladder
Trachea	Pancreas
Lungs and bronchi	Spleen
Heart	Kidneys
Thyroid	Adrenals
Parathyroids	Urinary bladder
Esophagus	Ovaries
Stomach	Uterus
Duodenum	Nasal cavity
Ileum	Brain
Colon	Pituitary
If required:	
Blood smear	Bone marrow smear
Eyes	Spinal cord

The animals have free access to food and water except during the exposure periods when the food is removed from the exposure as well as control chambers. This should prevent ingestion of UDMH absorbed by food. Cage areas are cleaned after the completion of the 6-hour exposure period and an acceptable air purge period. The experimental animals were randomly selected from the main group after quality control procedures and quarantine were completed and caged in conformance with ILAR standards for laboratory care.

All animals are observed hourly during exposure and will be observed daily during postexposure until termination of the study. The mice are weighed in groups on a monthly schedule with group mean weights followed.

RESULTS

The effect of UDMH exposure on mouse body weight is shown in Figure 4. After seven months of exposure, a difference in body weight gain in the exposed mice is apparent when compared to controls. The difference continues through the tenth month of exposure.

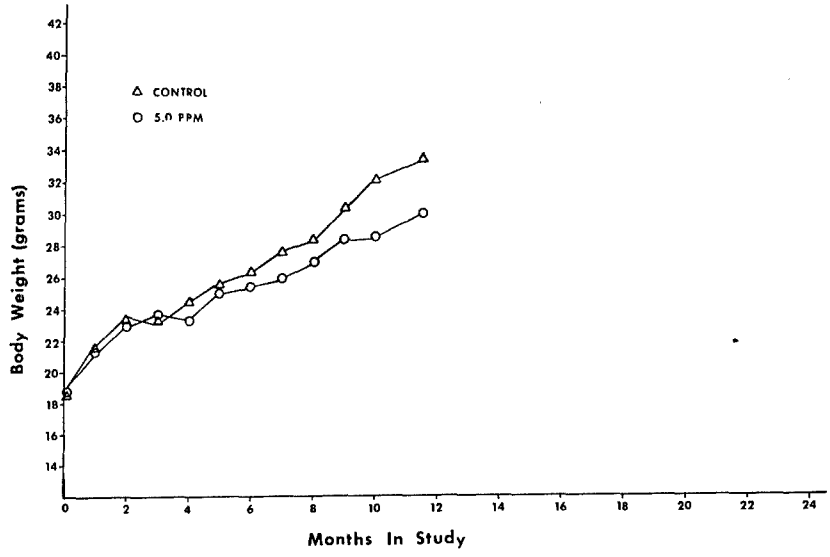


Figure 4. Effect of purified UDMH exposure on mouse growth.

There has been no significant effect of UDMH exposure on mouse mortality through the tenth month of exposure. Approximately 10% of the original 200 animals/group have died in both the control and 5 ppm UDMH exposure group. Histopathology reports from the dead animals are not yet available.

The exposure phase of the study is scheduled to conclude on 5 June 1979. The animals will then be removed for postexposure observation. Further information on the progress of this study will appear in future annual reports.

A STUDY OF THE ONCOGENIC CAPACITY OF INHALED HYDRAZINE AFTER CHRONIC LOW LEVEL EXPOSURE

The annual report of 1975 (MacEwen and Vernot) provided the complete details of the experimental protocol for the conduct of chronic inhalation studies to determine the oncogenic potential of hydrazine in four species of laboratory animals. The animal exposures were started during the summer and winter of 1975 and completed one year later at which time the extended postexposure phase of the study began to allow for possible tumor induction. The 1976, 1977, and 1978 annual reports (MacEwen and Vernot) contain a progressive accumulation of experimental data including mortality, body weight measurements, and clinical chemistry results for dogs tested during the 12 months of hydrazine exposure and through the postexposure portion of the study.

Except for the dogs, all animals are dead. The final postexposure sacrifice of rodents was done in March of 1978. The annual report of 1978 (MacEwen and Vernot) includes a discussion of the timing of the postexposure sacrifices.

This report sets forth current information on the health status of the dogs and, more importantly, histopathology results including tumorigenesis in rats and hamsters, as received from Huntingdon Research Center, England and the USAF School of Aerospace Medicine, VSD, Brooks Air Force Base, Texas, respectively.

As of 14 July 1979, all surviving dogs used in this study will have been held 36 months postexposure (48 months on test). The dogs were transferred from Brooks Air Force Base to the vivarium facility of the AMRL Veterinary Division on 4 April 1978. Comprehensive physical examinations are conducted every two months and clinical chemistry determinations are done semiannually.

Mentioned in previous annual reports (MacEwen and Vernot, 1977, 1978) was the observation of rectal bleeding from one dog in the 0.25 ppm exposure group at 4 months postexposure. A biopsy taken from a growth on the ventral surface of the rectum revealed a low grade adenocarcinoma. This diagnosis was confirmed in October of 1978 when the tumor was removed and examined histologically. The tumorous mass appeared to be confined to the mucosa. There was no evidence of invasion to normal tissue. The dog has been in good health since the surgery.

A control dog died on 9 May 1978. Death was due to respiratory failure because of the large amount of blood contained within the thoracic cavity. The extensive hemorrhaging into the thoracic cavity was the result of the rupture of numerous small capillaries that were being formed in response to pyogranulomatous reaction involving the lungs, pericardium and diaphragm. Bacteriology cultures isolated a Corynebacterium from the lesion.

The semiannual results of clinical chemistry determinations done in May 1978 revealed an elevated SGPT level for a dog in the 1.0 ppm N_2H_4 exposure group. A subsequent measurement in November 1978 showed a SGPT value of 228 International Units. A two-fold increase was seen in results two weeks later. The possibility of liver necrosis, cirrhosis, or tumor prompted the conduct of radiologic liver scan. Analysis of the results revealed, however, no changes consistent with the suspected abnormalities. Shortly after this examination, the SGPT value for this animal decreased to approximately 200 units and has remained at that level until May of this year when it again increased to 550 units. The dog appears to be in good health and a regimen of biweekly clinical chemistry determinations is being continued.

All other dogs in this study are currently healthy and clinical chemistry measurements fall within normal limits.

Groups of 100 male and 100 female Fischer 344 rats had received one year of intermittent, 6 hours/day, 5 days/week exposure to dose levels of 0.05, 0.25, 1.0 and 5.0 ppm hydrazine. The control group consisted of 150 male and 150 female Fischer 344 rats. An interim sacrifice was done at two years (one year postexposure). Forty rats (20 male and 20 female) from each exposure group, and 60 control (30 male and 30 female) were killed and submitted for necropsy. All surviving rats were held for observation, then sacrificed at $2\frac{1}{2}$ years ($1\frac{1}{2}$ years postexposure).

The histopathology results for rats are incomplete. However, the information from Huntingdon Research Center does provide the nonneoplastic and neoplastic histology for rats from a 2-year interim sacrifice and concentrates in detail on the findings of nasal tumors in rats that died or were sacrificed throughout the entire study.

NONNEOPLASTIC HISTOPATHOLOGY - RATS, 2-YEAR INTERIM SACRIFICE

In the respiratory system, varying degrees of acute inflammation were observed in the nasal cavity, larynx and/or trachea in some rats from the control and all treated groups. The incidence and severity of these changes were greatest in male (Table 10) and female (Table 11) rats from the group receiving 5.0 ppm, and in a proportion of these affected male rats, the acute rhinitis was associated with focal hyperplasia and/or squamous metaplasia of the nasal epithelium. In male rats from the group receiving 0.05 ppm, the incidence and/or degree of inflammation in the nasal cavity and larynx tended to be greater than in the controls and in male rats from the groups receiving 0.25 ppm or 1.0 ppm.

TABLE 10. SUMMARY OF NONNEOPLASTIC HISTOLOGIC FINDINGS IN THE RESPIRATORY SYSTEM OF CONTROL AND HYDRAZINE EXPOSED MALE RATS SACRIFICED AT TWO YEARS

NUMBER OF RATS WITH:	Control	0.05 ppm	0.25 ppm	1.0 ppm	5.0 ppm
Nasal Cavity					
Acute Inflammation					
Minimal	6	2	2		1
Slight		1	5	2	4
Moderate		5	1		5
Marked					1
Epithelial Hyperplasia					7
Squamous Metaplasia					5
Number of Rats Examined With Sections of Nasal Cavity	28	20	20	20	20
Larynx					
Acute Inflammation					
Minimal	3	3	1		2
Slight				2	1
Moderate		7	1		15
Squamous Metaplasia			1		1
Dilated Submucosal Glands	3	4	6	6	3
Prominent Lymphoid Follicles		2			1
Chronic Inflammation		1			
Number of Rats Examined With Sections of Larynx	29	18	19	17	20
Trachea					
Acute Inflammation					
Moderate					9
Dilated Submucosal Glands				1	
Epithelial Hyperplasia/ Squamous Metaplasia					1
Mineralization	25	17	14	18	15
Number of Rats Examined With Sections of Trachea	20	20	20	19	20

TABLE 11. SUMMARY OF NONNEOPLASTIC HISTOLOGIC FINDINGS IN THE RESPIRATORY SYSTEM OF CONTROL AND HYDRAZINE EXPOSED FEMALE RATS SACRIFICED AT TWO YEARS

NUMBER OF RATS WITH:	Control	0.05 ppm	0.25 ppm	1.0 ppm	5.0 ppm
Nasal Cavity					
Acute Inflammation					
Minimal	5	1	1		1
Slight	5	6	3	4	8
Moderate		2			3
Epithelial Hyperplasia/ Squamous Metaplasia		1			
Number of Rats Examined With Sections of Nasal Cavity	29	19	20	20	20
Larynx					
Acute Inflammation					
Minimal	3	1			
Slight	3		1	1	1
Moderate	2			1	11
Squamous Metaplasia	2		1	1	1
Dilated Submucosal Glands	7	3	2	4	1
Prominent Lymphoid Follicles					2
Number of Rats Examined With Sections of Larynx	27	15	17	18	19
Trachea					
Acute Inflammation					
Slight				1	2
Moderate					11
Dilated Submucosal Glands	1				
Mineralization	24	17	15	12	17
Number of Rats Examined With Sections of Trachea	30	20	19	20	20

There was evidence of the more severe grades of chronic respiratory disease in the lungs of a proportion of rats, particularly males, from the groups receiving 0.05 ppm or 5.0 ppm. The morphological changes included peribronchial/ peribronchiolar lymphoid hyperplasia, bronchiectasis/bronchiolectasis, acute pneumonitis, and bronchiectatic abscesses. The affected animals usually showed the more severe grades of acute inflammation in the nasal cavity, larynx, and trachea.

In the female reproductive system, acute endometritis was noted more frequently in rats from the group that had received 5.0 ppm hydrazine than in the controls. There were 8 cases in the 5 ppm group, one in the 1.0 ppm group, none in the 0.25 ppm and 0.05 ppm groups, and 2 in the controls. An additional noteworthy finding was acute salpingitis in the oviducts of 6 rats in the 5 ppm dose group. This lesion was not seen in any other exposed group or in controls. The number of rats actually examined in each group was exactly the same as the number sacrificed and mentioned previously.

Apart from the lesions discussed above, there is no evidence to indicate that any of the other morphological changes seen in the tissue examined could be attributed to hydrazine exposure. The nonneoplastic histopathology results for rats that died or were sacrificed before and after the two year interim kill are not available at this time.

NEOPLASTIC HISTOPATHOLOGY - RATS, 2-YEAR INTERIM SACRIFICE

Table 12 shows the incidence of nasal epithelial tumors in male rats while Table 13 shows the same for female rats. The majority of the tumors seen were benign, adenomatous nasal polyps. Additionally, small numbers of villous nasal polyps, muco-epidermoid papillomas, squamous cell papillomas, muco-epidermoid carcinomas and squamous cell carcinomas were encountered. Interestingly, a smaller number of tumors was found in rats that died or were sacrificed prior to the two-year stage of the study than was seen in the rats at the later time periods. This observation is the most prominent in the rats in the high dose group and can be seen if one calculates for each time period the percentage of tumors in animals examined. This gives a rough indication of the latency period for the induction of nasal tumors as a result of hydrazine exposure. The statistical test used to compare control tumor response with the exposed groups tumor response was the Fischer Exact Test. In the nasal tumor incidence tables for male and female rats, there are several cases of statistical differences between exposed and control groups. Tumor incidence overall and for the four time periods is significant for male and female rats in the 5 ppm, high dose group. The only exception is in the D1 group, female rats that died during the first 24 months of the study. At the 1.0 ppm level, significance is obtained for overall tumor incidence for male and female rats, but occurs only during one time period (D2) for male rats that died between interim sacrifice and study termination. Although there were a few tumors seen in rats in the 0.05 and 0.25 ppm exposure groups, the incidence was too small to attach any statistical significance.

TABLE 12. INCIDENCE OF NASAL EPITHELIAL TUMORS IN CONTROL AND HYDRAZINE EXPOSED MALE FISCHER 344 RATS

	Control				0.05 ppm				0.25 ppm				1.0 ppm				5.0 ppm			
EPITHELIAL TUMOR	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T
Single Benign	-	-	-	-	-	1	-	1	-	-	1	1	-	2	5	2	2	6	14	3
Multiple Benign	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	7	15	7
Malignant	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	3#	2	2#
Number of Rats Examined With Section of Nasal Cavity	50	28	58	11	27	20	37	14	33	20	29	14	19	20	43	15	29	20	36	14
% of Rats With Nasal Tumors	0	0	0	0	0	5	2.7	7.1	0	0	3.4	7.1	0	10	14**	13.3	41.3**	80**	80.5**	85.7**
% Overall Total of Rats With Nasal Tumors	0				3				2				10.3**				69.6**			

D1 = Rats dying during first 24 months.
I = Rats killed at 2-year interim sacrifice.
D2 = Rats dying between interim sacrifice and termination.
T = Rats killed at terminal sacrifice.

= Includes 1 rat with additional benign tumor.
+ = Squamous cell papilloma in nasolachrymal duct.
** = Significant at the 0.1 level as determined using the Fischer Exact Test.

TABLE 13. INCIDENCE OF NASAL EPITHELIAL TUMORS IN CONTROL AND HYDRAZINE EXPOSED FEMALE FISCHER 344 RATS

	Control				0.05 ppm				0.25 ppm				1.0 ppm				5.0 ppm			
EPITHELIAL TUMORS	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T	D1	I	D2	T
Single Benign	-	-	-	-	1	-	-	-	-	-	-	-	1	1	2	-	2	6	9	4
Multiple Benign	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	3	3
Malignant	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3#	2##
Number of Rats Examined With Section of Nasal Cavity	49	29	47	19	39	19	27	14	35	20	24	19	20	20	42	13	30	20	29	15
% of Rats With Nasal Tumors	0	0	0	0	2.5	0	0	0	0	0	0	0	5	5	4.7	0	10	35**	51.3**	60**
% Overall Total of Rats With Nasal Tumors	0				1				0				4.2*				36.1**			

D1 = Rats dying during first 24 months.
I = Rats killed at 2-year interim sacrifice.
D2 = Rats dying between interim sacrifice and termination.
T = Rats killed at terminal sacrifice.

= Includes 1 rat with additional benign tumor.
= Includes 2 rats with additional benign tumors.
* = Significant at the 0.05 level as determined using the Fischer Exact Test.
** = Significant at the 0.01 level as determined using the Fischer Exact Test.

In review of the foregoing information and tables for incidence of nasal tumors in rats exposed to 4 dose levels of hydrazine, the following pertinent comments are made:

1. There are indications of a gradation in latency period for induction of tumors, i.e., there was a larger incidence seen in the 5 ppm exposed rats examined at two years to study conclusion.

2. Statistically significant tumor incidence was seen at highest hydrazine exposure levels, 1 and 5 ppm. A dose response relationship is indicated.

3. There were no nasal tumors in control rats.

4. Male rats were more severely affected than female rats, i.e., the incidence of tumors was greater in male than female rats; and tumors tended to occur earlier in the study in male rats than in female rats.

5. Until the nonneoplastic histology information is available and examined for all rats, no conclusions should be drawn regarding the possible association of nasal tumors and acute rhinitis.

Apart from the information on nasal tumors, an examination of other individual tumor types seen in male and female rats at the 2-year interim sacrifice revealed no evidence of excess tumor production in exposed animals. The distribution and types of tumors were within the range for rats of the Fischer 344 strain. Tumor findings for time periods other than the 2-year period are not available at this time. All of this information will be presented in subsequent reports.

Groups of 200 male Golden Syrian hamsters had received one year of intermittent, 6 hours/day, 5 days/week exposure to dose levels of 0.25, 1.0 and 5.0 hydrazine. The control group also consisted of 200 animals. All surviving animals were held for one year postexpsure, then sacrificed.

Complete histopathology results for hamsters were received from the Veterinary Science Division at Brooks Air Force Base where the tumor and nontumor nomenclature was developed and used for automated data processing of the results for hamsters. Tumor incidence tables and statistical analysis, using the Fischer Exact Test were done by the THRU.

NEOPLASTIC HISTOPATHOLOGY - HAMSTERS

Table 14 shows tumor incidence in the various groups of exposed and control hamsters. The outstanding finding in hamsters is a statistically significant increase in benign nasal polyps. These tumors were seen in 16 of the 5 ppm exposed animals, while only one was noted in the control group. The only other tumor types of possible importance are those of the colon in the 5 ppm exposure group. There were 3 primary adenocarcinomas, one benign leiomyoma and one benign papilloma. When these tumor types were separately subjected to the Fischer Exact Test, none showed statistical significance. However, the sum of all five did show statistical significance. There was a rather large incidence of cortical adenomas found in the adrenals of all groups of exposed hamsters but with no statistical validity in that the incidence was actually greater in the control group. This type of tumor is commonly seen in aged hamsters. Incidence of other tumors in the various organs was low. No biological significance is attached to the statistical finding noted for benign thyroid adenomas in the 0.25 ppm hydrazine exposure group.

TABLE 14. TUMOR INCIDENCE IN CONTROL AND HYDRAZINE EXPOSED MALE GOLDEN SYRIAN HAMSTERS†

TUMOR TYPE	UNEXPOSED CONTROLS	0.25 PPM EXPOSED	1.0 PPM EXPOSED	5 PPM EXPOSED
Nares, Trachea, Bronchi				
Polyp (B)	1/181	0/154	1/148	16/160**
Basal Cell (P)	0/181	0/154	1/148	0/160
Basal Cell (B)	0/181	0/154	0/148	1/160
Adenoma (P)	0/181	1/154	0/148	0/160
Adenoma (B)	0/181	0/154	0/148	2/160
Total Tumors	1/181	1/154	2/148	19/160**
Lung				
Bronchogenic Adenoma (P)	1/179	0/154	1/146	0/155
Bronchogenic Adenoma (B)	0/179	0/154	0/146	2/155
Liver				
Reticulo-endotheliomas (B)	1/180	0/160	0/148	0/159
Spleen				
Hemangioma (P)	1/160	1/129	0/130	2/138
Reticulo-endotheliomas (P)	1/160	2/129	0/129	0/138
Reticulo-endotheliomas (B)	1/160	0/129	0/129	0/138
Bone Marrow, Blood				
Myelogenous (P)	0/157	0/134	1/136	0/135

TABLE 14. TUMOR INCIDENCE IN CONTROL AND HYDRAZINE EXPOSED MALE GOLDEN SYRIAN HAMSTERS† (Continued)

TUMOR TYPE	UNEXPOSED CONTROLS	0.25 PPM EXPOSED	1.0 PPM EXPOSED	5 PPM EXPOSED
Bone				
Osteoma (P)	0/177	0/152	0/148	1/156
Lymph Nodes				
Reticulo-endotheliomas (P)	5/167	4/143	5/140	6/146
Reticulo-endotheliomas (B)	0/167	1/143	0/140	0/146
Kidney				
Renal Adenoma (P)	1/179	2/164	0/145	0/160
Reticulo-endotheliomas (B)	1/179	0/164	0/145	0/160
Thyroid				
Adenoma (P)	1/145	1/117	0/127	0/137
Adenoma (B)	0/145	4/117*	1/127	0/137
"C" Cell Adenoma (P)	0/145	1/117	0/127	0/137
"C" Cell Adenoma (B)	0/145	0/117	0/127	4/137
Parathyroid				
Adenoma (B)	3/111	3/88	2/82	2/100
Adrenal				
Cortical Adenoma (B)	40/177	18/155	19/141	23/153
Cortical Adenoma (P)	6/177	5/155	3/141	4/153
Stomach				
Papilloma (B)	0/169	1/149	0/140	0/145
Basal Cell (P)	0/169	0/149	2/140	1/145
Pleura, Peritoneum Mesenteries				
Fibroma (P)	0/161	2/152	0/139	0/147
Pancreas				
Islet Cell Adenoma (B)	0/114	0/98	0/99	1/107
Small Intestine				
Adenocarcinoma (P)	1/148	1/140	0/132	0/141
Colon				
Adenocarcinoma (P)	0/158	0/146	2/129	3/139
Leiomyoma (B)	0/158	0/146	0/129	1/139
Papilloma (B)	0/158	0/146	0/129	1/139
Total Tumors	0/158	0/146	2/129	5/139**

TABLE 14. TUMOR INCIDENCE IN CONTROL AND HYDRAZINE EXPOSED MALE GOLDEN SYRIAN HAMSTERS† (Continued)

TUMOR TYPE	UNEXPOSED CONTROLS	0.25 PPM EXPOSED	1.0 PPM EXPOSED	5 PPM EXPOSED
Skin				
Leiomyoma (B)	0/170	1/161	0/146	0/147
Squamous Cell Carcinoma (P)	0/170	1/161	0/146	0/147
Trichopithelioma (B)	0/170	1/161	0/146	0/147
Hemangioma (B)	0/170	0/161	1/146	0/147
Fibroma (B)	0/170	0/161	0/146	1/147
Pituitary				
Adenoma (B)	0/163	1/133	0/129	1/138

† - Metastatic tumors in various organs were not counted.
(P) - Primary malignant tumors.
(B) - Benign tumors.
* - Significant at the 0.05 level as determined using the Fischer Exact Test.
** - Significant at the 0.01 level as determined using the Fischer Exact Test.

NONNEOPLASTIC AND PRENEOPLASTIC HISTOPATHOLOGY - HAMSTERS

The nonneoplastic histopathology data for exposed hamsters included descriptions and discussion of many lesions which occurred as frequently as or more frequently than in control animals. These were probably the result of aging or of chronic disease states to which hamsters are susceptible. Analysis of the incidence of such lesions would not elucidate the effect of hydrazine exposure on target organs. Therefore, the data were examined to select specific organ lesions which might have been related to exposure. This preliminary examination indicated that lesions in the nares, trachea and bronchi (considered as one organ in the accounting), lung, liver, spleen, lymph nodes, kidney, thyroid, adrenal, colon, and testes might have occurred more frequently in exposed animals and could be possible sites of toxic action by hydrazine.

The next consideration was a more specific examination of the incidence of each type of lesion recorded for each of the 10 organs to permit percentage as well as statistical comparisons of the differences between each exposed group and between each exposed group and control. This information is presented in Table 15 and shows incidence percentages as well as statistical significance.

TABLE 15. NONNEOPLASTIC HISTOLOGIC FINDINGS IN ORGANS OF CONTROL AND HYDRAZINE EXPOSED MALE HAMSTERS

LESION DESCRIPTION	UNEXPOSED CONTROL	0.25 PPM EXPOSED	1.0 PPM EXPOSED	5 PPM EXPOSED
Nares, Trachea, Bronchi				
Submucosal cysts	30/181(17)	29/154(19)	29/148(20)	36/160(23)
Rhinitis	4/181(2)	6/154(4)	9/148(6)	3/160(2)
Hyperplasia	0/181(0)	0/154(0)	2/148(1)	2/160(1)
Squamous Metaplasia	0/181(0)	0/154(0)	0/148(0)	4/160(3)
Lung				
Adenomatosis	15/179(8)	22/154(14)	28/146(19)**	21/155(14)
Interstitial Pneumonitis	28/179(16)	30/154(20)	38/146(26)*	27/155(17)
Bronchiolar Hyperplasia	2/179(1)	2/154(1)	3/146(2)	4/155(3)
Liver				
Amyloidosis	42/180(23)	67/160(42)**	68/148(46)**	79/159(50)**
Hemosiderosis	42/180(23)	63/160(39)**	77/148(52)**	94/159(59)**
Bile Duct Hyperplasia	14/180(8)	31/160(19)**	28/148(19)*	44/159(28)**
Biliary Cyst	45/180(25)	45/160(28)	42/148(28)	55/159(35)*
Spleen				
Amyloidosis	39/160(24)	39/129(30)	57/130(44)**	60/138(44)**
Lymph Nodes				
Lymphadenitis	6/167(4)	13/143(9)*	17/140(12)**	16/146(11)**
Lymphoid Hyperplasia	15/167(9)	18/143(13)	15/140(11)	6/146(4)
Kidney				
Interstitial Amyloidosis	15/179(8)	19/164(12)	21/145(15)	28/160(18)**
Glomerular Amyloidosis	39/179(22)	53/164(32)*	67/145(46)**	77/160(48)**
Mineralization	55/179(31)	78/164(48)**	51/145(35)	82/160(51)**
Thyroid				
Amyloidosis	9/155(6)	20/117(17)**	11/127(9)	22/137(16)**
Adrenal				
Amyloidosis	38/177(22)	49/155(32)*	52/141(37)**	76/153(50)**
Degeneration	25/177(14)	29/155(19)	26/141(18)	34/153(22)*
Hemosiderosis	3/177(2)	8/155(5)	7/141(5)	7/153(5)
Colon				
Colitis	10/148(7)	17/146(12)	13/129(10)	20/139(14)*
Testis				
Senile Atrophy	33/185(18)	41/160(26)	40/149(27)*	55/159(35)**
Aspermatogenesis	27/185(15)	20/160(13)	18/149(12)	36/159(23)*
Hypospermatogenesis	33/185(18)	35/160(22)	38/149(26)	41/159(26)

( ) - % incidence, rounded off to whole numbers.
* - Significant at the 0.05 level as determined using the Fischer Exact Test.
** - Significant at the 0.01 level as determined using the Fischer Exact Test.

Two important observations emerge from Table 15. Degenerative disease, characterized by amyloidosis in the livers, spleens, kidneys, thyroids, adrenals; and liver hemosiderosis, kidney mineralization, general degeneration of the adrenals; and senile atrophy, aspermatogenesis and hypospermatogenesis, is a common finding in all groups of hamsters. The important fact is that these lesions occur with statistically significantly higher frequency in the exposed groups; and in most cases, a dose response relationship can be seen. The implication is that the stress of 12 months of hydrazine exposure at the various dose levels used tended to hasten the aging - degenerative process in a dose dependent manner.

Morphologic changes such as nasal hyperplasia and squamous metaplasia, especially the latter, could be considered as preneoplastic and related to the findings of nasal tumors previously described. Although there was no significant tumor incidence in the lungs of exposed hamsters (Table 14), the findings of adenomatosis and bronchiolar-hyperplasia could also be considered as preneoplastic tissue change. The cases of lymphoid hyperplasia are more numerous in the 0.25 and 1.0 ppm exposure groups than in control but are without statistical significance.

Histopathology evaluation for the mice used in this study has not yet been received from the Huntingdon Research Center.

1,2-DIMETHYLHYDRAZINE DIHYDROCHLORIDE AS A POSITIVE TUMORIGENIC CONTROL FOR THE EXPERIMENTAL ANIMAL SPECIES USED IN CURRENT ONCOGENIC STUDIES

1,2-Dimethylhydrazine dihydrochloride (SDMH dihydrochloride) has been proven a tumorigenic agent to rodents when given by oral or subcutaneous administration. Toth and Wilson (1971) gave SDMH dihydrochloride in the drinking water of mice for their lifetimes. Ninety-two percent of male and 98% of females had angiosarcomas, mainly localized

in the muscle, liver and pararenal tissues after median latent periods of 42 and 45 weeks. In addition, 24% of males and 44% of the females had lung adenomas after similar latent periods. Oral doses to rats (Druckrey et al., 1967; Druckrey, 1970) produced adenocarcinomas of the colon and rectum.

Hawks et al. (1971, 1972) reported subcutaneous injections of SDMH dihydrochloride to mice at doses of 15 mg/kg per week for a total of 22 weeks developing tumors in the descending colon and rectum in 52 of 58 surviving mice. Weekly subcutaneous injections, reported by Thurnherr et al. (1973), of 20 mg/kg for 2 to 24 weeks to CF_1 mice produced multiple carcinomas of the colon in 90% of the animals. The earliest tumor was found in a mouse that died at 135 days.

Previous oncogenic studies done in this laboratory on the hydrazine compounds utilized specific strains of the various rodent species. These were female C57Bl/6 mice, male and female Fischer 344 rats and male Golden Syrian hamsters.

In order to verify that the animals supplied to us responded to a known carcinogen, this study was designed to serve as a positive control for a concurrent monomethylhydrazine inhalation study.

The 1978 annual report (AMRL-TR-78-55) defines the materials and methods used in this study as well as the details concerning the subcutaneous rangefinding LD_{50}'s done on each species of animal. The rangefinding LD_{50}'s and 95% confidence limits are shown below:

Mice:	38.2 (34.6-42.4) mg/kg
Hamsters:	50.0 (44.4-56.0) mg/kg
Rats, Male:	121.8 (70.4-158.4) mg/kg
Rats, Female:	125.8 (93.1-169.8) mg/kg.

The toxic response of the hamsters was very similar to that in the mouse. The rats, however, were more resistant with no difference noted between the sexes.

Initially, 20 mg/kg was selected as a weekly dose which should have produced tumors without causing significant early mortality. The hamsters were unable to tolerate a weekly dose of 20 mg/kg, and all died within fifteen weeks. Therefore, it was necessary to lower the dose for hamsters to 10 and 5 mg/kg to give the doses shown in Table 16 for all species. Mean body weight effects were noticed immediately in the hamsters and male rats but not in female rats when compared to their respective controls (Figures 5-7). The 10 mg/kg dosed hamster group showed a dramatic decrease in mean body weight following 16 weekly injections. The 5 mg/kg group did not show the sharp decline in mean body weight although a statistically significant difference from the controls existed from the 13th week to the conclusion. The mean body weights of the female rat group did not show any difference from controls until after 36 weekly injections.

TABLE 16. MORTALITY RESPONSE TO WEEKLY SUBCUTANEOUS INJECTONS OF SDMH DIHYDROCHLORIDE

Species & Sex	Original Number	Weekly Dose (mg/kg)	Weeks to First Death	Weeks To 90% Mortality
Rats, ♂	25	20	19	39
Rats, ♀	25	20	25	51
Mice, ♀	50	20	25	44
Hamsters, ♂	50	10	19	33
Hamsters, ♂	50	5	24	52*

*Study terminated at one year with 84% mortality.

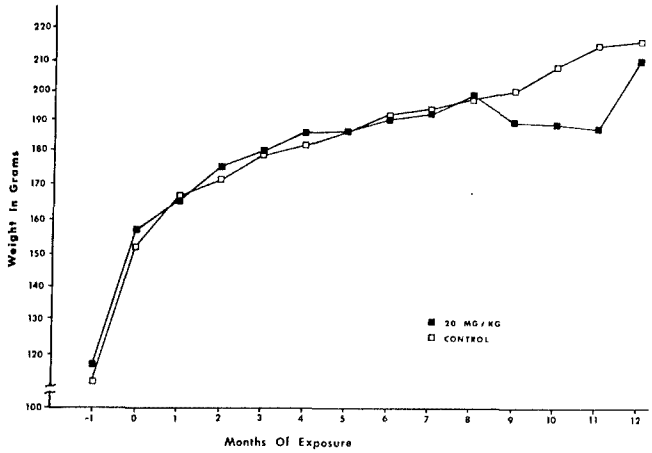


Figure 5. The effect of SDMH on female Fischer 344 rat growth.

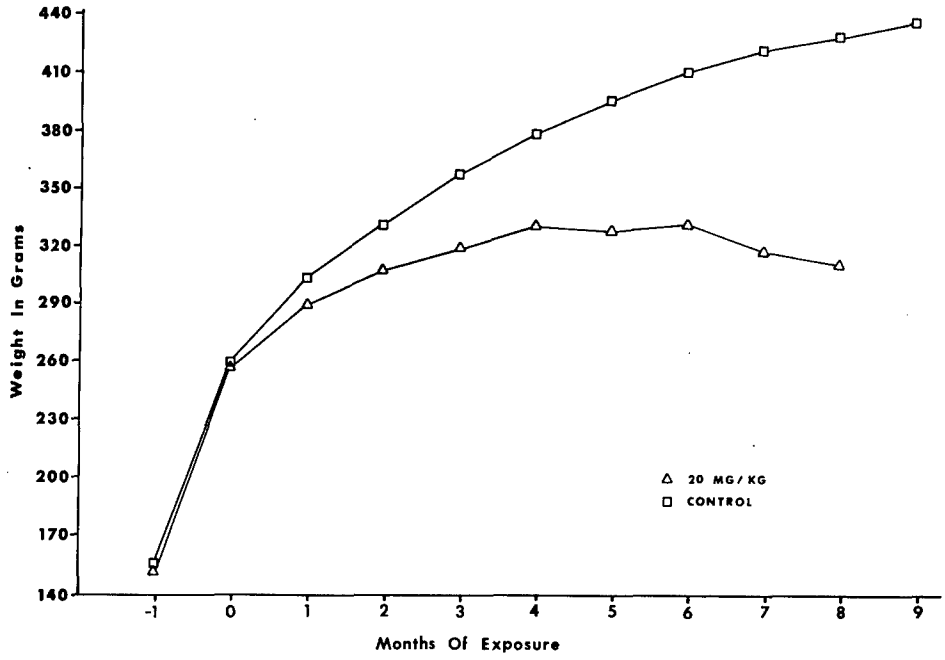


Figure 6. The effect of SDMH on male Fischer 344 rat growth.

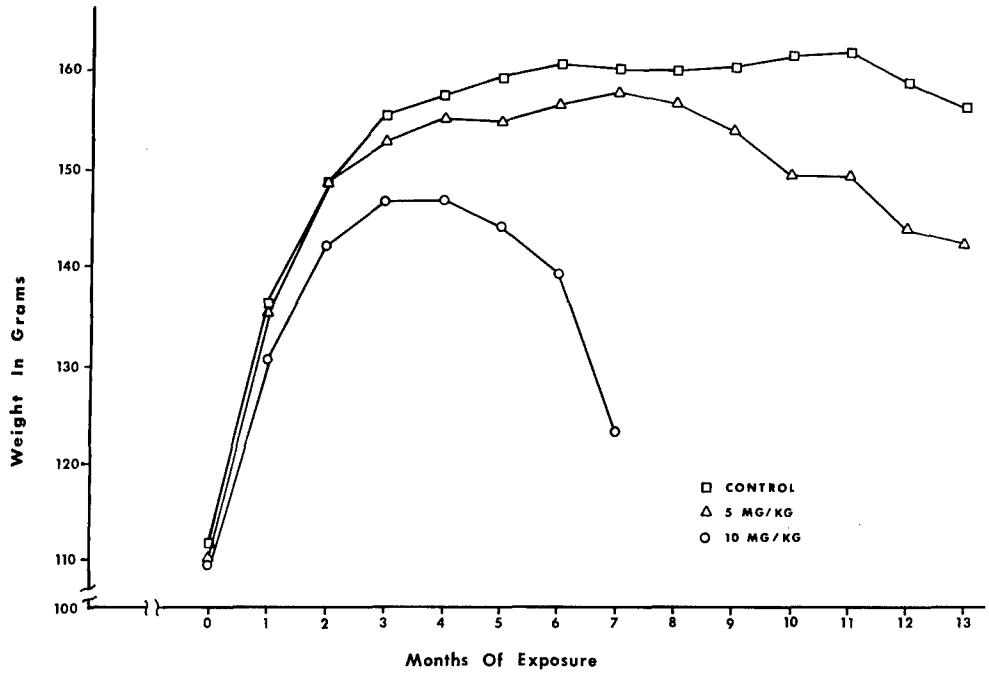


Figure 7. The effect of SDMH on male Golden Syrian hamster growth.

Table 16 summarizes mortality effects, and Figure 8 shows cumulative mortality curves for all species and doses. Male rats are obviously much more susceptible than are the female rats. The mice are less susceptible than the male rats but more than the female rats.

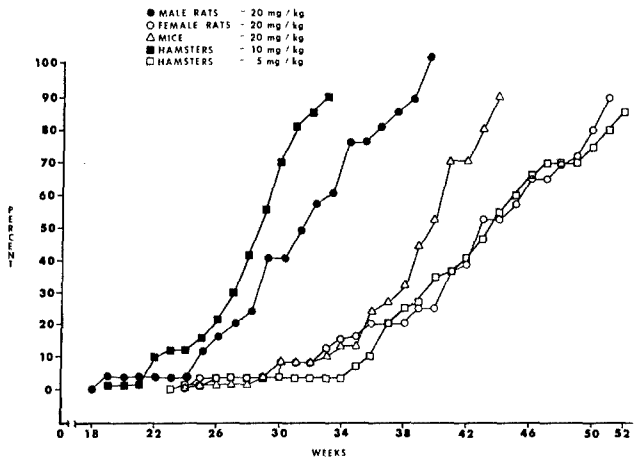


Figure 8. Cumulative mortality in SDMH dihydrochloride dosed animals.

The most sensitive species was the hamster. The group receiving one-half of the dose the mice and rats received attained 90% mortality in a shorter time span than the male rats. The group receiving one-quarter of the dose followed a similar mortality pattern as the female rats. Control animal mortality was not significant, never exceeding 14% up to the time of sacrifice.

Histopathology examinations have been completed on the mice and both sexes of rats. The hamsters that were recently sacrificed are still being examined, and the results will not be completed in time to be included in this report.

Injection of mice with SDMH produces several significant nonneoplastic lesions. These include splenic extramedullary hematopoiesis, thymic atrophy, biliary hyperplasia, hepatocytomegaly, adrenal cortical megalocytosis, and ovarian vascularization.

Splenic extramedullary hematopoiesis, involving primarily the red blood cell series, indicates that injection of SDMH in some way produces an increased demand for red blood cell production. The mechanism of this is not evident from the necropsy material. Thymic necrosis and atrophy in this study were noted only in test animals; however, most of these were younger than the controls at death. Thus, SDMH appears to induce this lesion. Premature thymic atrophy could markedly alter the immune response of injected mice.

SDMH exposure induces proliferation of small intrahepatic bile ductules in test mice. This is a fairly common reaction of the liver to irritant materials which are excreted through the bile. SDMH also induces a marked alteration in the appearance of hepatocytes and of adrenal cortical cells. The most striking changes are within the nucleus, although the cytoplasm is altered as well. Scattered individual cell necrosis occurs in both organs.

A change described as vascularization of the ovary is sometimes seen in older mice, but in this case the lesion appeared only in test animals. The ovarian stroma seems to disappear and be replaced by large dilated blood-filled capillary spaces. This change appears to become so extensive that it can result in a single large blood-filled space or hematoma. Necrosis of the ovarian stroma is not seen, nor does the vascular endothelium appear proliferative.

Only exposed mice had inflammation within the nasal sinuses, yet approximately the same number of test and control animals had inflammatory lesions within the lung. There was decidedly less perivascular cuffing within the lungs, salivary glands, liver, kidneys, and urinary bladder in injected mice than in control mice. Perivascular cuffing ordinarily increases with age, and this may merely reflect the older age of many of the controls. This may also be related to lymphoid destruction similar to that seen in the thymus. Lymphoid necrosis was not evident in the lymph nodes, however.

SDMH induces malignant neoplasms of the vascular endothelium and of intrahepatic bile ducts in C57Bl/6 female mice, as shown in Table 17. Benign neoplasms were induced in the colonic mucosa. Whether or not the single examples of three other benign neoplasms were induced by SDMH or not is difficult to determine. The alveolar/bronchiolar adenomas and pituitary adenomas which were noted in the controls are neoplasms which are commonly present in old mice. Presumably, they did not occur in the test mice because most of them died before such neoplasms began to appear.

TABLE 17. SUMMARY OF NEOPLASTIC LESIONS IN SDMH TREATED C57B1/6 FEMALE MICE

	Control	Test
Circumanal Gland Adenoma	0	1
Nasal Papilloma	0	1
Alveolar/Bronchiolar Adenoma	2	0
Hemangiosarcoma	0	25
Cholangiocarcinoma	0	10
Hepatocellular Adenoma	0	1
Small Intestinal Papilloma	1	0
Colonic Papilloma	0	8
Malignant Neoplasm (Unidentified)	0	5
Pituitary Adenoma	10	0
TOTALS	13	51

Of the 25 male and 25 female rats assigned to each of the two groups, one test group and one control group, all of the female rats were submitted for histopathologic examination while only 24 males each were submitted for histopathologic examination from the two groups.

The most prominent finding in this study is the presence of a high number of malignant neoplasms seen in the test group; 72 malignant neoplasms were seen in the test group, none were seen in the controls. Forty-one malignancies are deemed carcinomas of various types, all of which appeared to arise in mucosal epithelium of the gastrointestinal system. There were also 26 malignant neoplasms seen in Zymbal's gland.

Significant nontumor pathologic changes were seen in the gut, liver, and lymphoid system of the test group. Seven intussusceptions were seen in the bowel, many of which were caused by the neoplasms seen there. There were 15 animals which had dysplastic enlargement of liver cord cells (hepatocytomegaly). Lymphoid depletion was evident in lymphoid organs throughout the exposed animals.

It is evident from the results of the histopathological examination that male and female Fischer 344 rats and female C57B1/6 mice will show malignant tumors when exposed to a known carcinogen. Therefore, the strains of rats and mice currently being used in the long-term oncological studies should show positive results if the compound to which they are being exposed is a carcinogen.

The results of the histopathologic examination of the hamsters will not be available in time for this report. However, these results will be reported in the next annual report.

CHRONIC EFFECTS OF LOW LEVEL INHALATION EXPOSURES TO FLUOMINE PARTICULATES

The compound fluomine [colbalt-bis (3-fluorosalicyl-aldehyde)-ethylene diimine], when activated, is capable of selectively absorbing oxygen from the air and upon heating will release pure molecular oxygen. This oxygen-scavenging property renders it useful as a possible component in life support systems for high altitude aircraft flights.

MATERIAL AND METHODS

Preliminary studies of two-week duration as well as 6-month chronic inhalation exposures were described in two previous annual reports (MacEwen and Vernot, 1977 and 1978). Included in the earlier reports were experimental data obtained during a 6-month exposure of animals to 0.1 and 0.5 mg/m^3 fluomine. Also included were data concerning the gross pathology observations on animals sacrificed immediately following exposure as well as mean body weight effects on animals held postexposure. The animal groups for each exposure concentration and controls consisted of 100 male Sprague-Dawley rats, 140 female CF-1 mice, 24 male Hartley guinea pigs, and 8 beagle dogs. The exposures were conducted 6 hours each day, 5 days per week. After a reiteration of the generation and analysis methods used in this study, this report will deal only with data accumulated since the last report.

The fluomine particulates, produced by a Wright Dust Feeder®, were generated into a 200 liter mixing chamber prior to being drawn into the exposure domes by negative pressure. Regulation of the dust feeder gear ratios and/or the air passing through the mixing chamber controlled the concentration as well as the particle size entering the chamber.

Analysis of the fluomine concentration was accomplished by taking hourly filter samples for colorimetric analysis. The fluomine samples were dissolved in 1N HNO_3 and absorbance at 365 nm measured using a GCA McPherson spectrophotometer. Checks were made by counting the particles in the 1.4-3.0 micron range using the Royco analyzer. Since the greater part of the mass of the fluomine was in this size range, fluctuations in chamber concentrations could be easily detected by changes in the channel output representing this range.

An interim sacrifice of animals (MacEwen and Vernot, 1978) took place 12 months postexposure, at which time all surviving guinea pigs and one-half of the surviving rats and mice were sacrificed. The remaining animals were held for an additional six months and then sacrificed. The numbers of rats and mice alive at the conclusion of the study are shown below:

Fluomine Concentration (mg/m^3)	Male Rats	Female Mice
0.5	15	41
0.1	11	35
0.0 (controls)	13	29

Gross examination of the animals sacrificed after 12 and 18 months of postexposure observation showed no apparent dose-related lesions. Histopathologic results are not yet available; therefore, any conclusion or prediction of safe levels will have to wait until that report is available.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 JET FUEL

JP-10 is a synthetic saturated polycyclic hydrocarbon. It is being utilized as a jet fuel either alone or as a major constituent (70%) of JP-9 fuel because of its high density and other desirable properties. In the latter application, it has been substituted for RJ-4 which is a reduced dimer of methylcyclopentadiene. The chronic inhalation toxicity of RJ-4, and RJ-4 in combination with RJ-5, another constituent of JP-9 fuel, was detailed in previous annual reports (MacEwen and Vernot, 1974, 1975, 1976).

JP-10 is a single chemical entity identified as tricyclo-($5.2.1.0^{2,6}$) decane. Gas chromatographic analysis of two drums of this fuel in use indicated that it is 98% pure JP-10 and 2% miscellaneous unidentified impurities. Attempts to identify the impurities are in progress. The results will allow assessment of any potential impact on toxic response of animals in the 12-month study reported here.

The quantities of JP-10 needed for the experiment were supplied by the Air Force. The known physical properties of JP-10 are shown below:

Molecular weight:	136
Boiling point:	360 F
Density, 70 F:	0.940
Viscosity, 70 F:	3.5
Flash point:	135 F
Saturated vapor concentration:	∿ 1500 ppm.

Since no information appears in the literature concerning the toxicologic properties of JP-10, a series of studies was planned beginning with acute studies, including emergency exposure limit tests and culminating in a long-term chronic study. The experiments were conducted to develop the necessary data for hazard evaluation and establishment of safe exposure limits as well as to identify the oncogenic potential of JP-10 fuel.

Preliminary acute inhalation experiments had shown that mice were the most sensitive species to JP-10 with all of 6 animals exposed to 1000 ppm dying within 4 hours. To aid in selection of a concentration of JP-10 suitable for use in a year long, 6 hours/day, 5 days/week exposure regimen, groups of 5 female rats and 5 female mice were exposed to 250 ppm for five 6-hour exposure days. The coordination of the mice appeared slightly affected on the first day of exposure. Respiration rates of both rats and mice were more rapid than normal during the second day's exposure. One mouse had a slight convulsion early in the second exposure day but recovered and appeared normal thereafter. For the rest of the exposure, no further signs of toxic stress were noted in either species. Mean body weights of the mice did not increase during the week following termination of exposure. A detailed report of all acute inhalation experiments, oral and intraperitoneal toxicity, dermal and ocular irritation tests, and sensitization studies can be found elsewhere in this report.

As a result of the toxic effects shown in mice in the short-term inhalation tests, an exposure concentration of 100 ppm JP-10 was selected for chronic exposure of animals to determine safe exposure limits.

The experimental animals used in this study were randomized from the main groups after quality control procedures and quarantine periods were completed. Group assignments of each species were made by the use of the THRU Computer Program RANDUM which utilizes the FORTRAN library subroutine RANF(X).

Each exposure chamber contains as few species as possible to minimize the risk of cross infection. Therefore, dogs and rats are housed in one chamber and mice and hamsters in a companion chamber. The numbers of animals in each chamber and cage are compatible with ILAR standards for animal care. The numbers of rodents permit a statistically valid number of each species to reach the required age for tumor induction with natural and toxicologic attrition. Purebred beagle dogs were selected from a baseline group on the basis of examination and general observation of good health and several preexposure clinical chemistry determinations. Fischer 344 rats and Golden Syrian hamsters were obtained from the Charles River Breeding Laboratories. C57Bl/6 mice were purchased from the Jackson Laboratory. Distribution of the animal groups and other pertinent information is shown below:

Species, Sex	Chamber 1	Chamber 2	Unexposed Controls
Rats, male	-	50	50
Rats, female	-	50	50
Mice, female	200	-	200
Hamsters, male	100	-	100
Dogs, male	-	4	4
Dogs, female	-	4	4

All animals are observed hourly during the exposure phase of the study. Daily observations will be conducted during the postexposure phase until termination of the experiment. Exposure to JP-10 will last for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off to simulate a human exposure regimen.

Food is provided to the animals during the nonexposure time periods, and the chambers are cleaned daily following the completion of the 6-hour exposure and minimum 30-minute air purge period. Analysis of chamber concentration is used to verify the adequacy of the purge time.

Rats, hamsters and dogs are being weighed individually at biweekly intervals during exposure and will be weighed monthly during the postexposure period. Mice are weighed in groups with group mean weights followed on a monthly basis throughout the experimental period.

Blood samples are drawn from all dogs at biweekly intervals and clinical determinations using the following battery of tests:

HCT	Potassium
HGB	Calcium
RBC	Albumin/Globulin
WBC	Total Protein
Differential Cell Counts	Glucose
MCV	Alkaline Phosphatase
MCH	SGPT
MCHC	SGOT
Sodium	Bilirubin
BUN	Creatinine.

Following the one-year exposure period, 20 mice/group and 10 hamsters/group will be necropsied to determine chronic exposure effects while the remaining rodents will be held for a year of postexposure observation or until cumulative mortality exceeds 90%. The dogs will be held for postexposure observation for five years during which time they will receive quarterly physical examination and semi-annual blood

All animals that die or are sacrificed in these studies shall be necropsied. The necropsy is defined as external examination, including body orifices, and examination and fixation of 33 tissues using the NCI protocol for oncogenic screening.

The exposure chambers are operated with nominal airflows of 30 cfm at a slightly reduced pressure, 725 mm Hg to avoid leakage of JP-10 vapor.

A Buchler Polystaltic® Pump is used to deliver the liquid JP-10 from a storage drum into a spiral evaporator where it is vaporized and introduced into the chamber air supply system through a 1/4" stainless steel line. The exposure dome concentrations are monitored using a Beckman Model 400 Hydrocarbon Analyzer. Sequential sampling is conducted on the pair of chambers.

Exposures began on 5 June 1978 and have been in progress approximately 10-1/2 months at the time this report was prepared. The monthly mean chamber concentrations of JP-10 are given in Table 18.

TABLE 18. JP-10 EXPOSURE CONCENTRATIONS PRESENTED TO EXPERIMENTAL ANIMALS. MEAN MONTHLY CONCENTRATIONS IN PARTS PER MILLION.

Month	Chamber 5	Chamber 6
June	99.2	100.0
July	99.4	99.5
August	100.8	98.9
September	101.4	101.1
October	100.3	100.3
November	99.3	100.0
December	99.4	99.7
January	99.8	100.5
February	99.6	99.7
March	100.4	99.9

Very few signs of toxic stress have been observed since the beginning of the animal exposures to 100 ppm JP-10. Exposed dog and mouse weights are unaffected thus far. Mean body weights for groups of exposed and control male rats, female rats, and male hamsters collected on a biweekly schedule through 44 weeks of the study are shown in Figure 9. Weights of male rats are depressed as a result of JP-10 exposure. They are statistically different from control values at all time periods. Mean weight differences of these rats compared with controls are as large as 35 grams from 34 through 44 weeks. Exposed female rat weights, oddly, show a somewhat greater weight gain than their controls throughout most of the study so far. Although this response has been extensively investigated in regard to weighing procedures, animal care, and other possible variables, the reason for the response remains unexplained. The growth rate of exposed hamsters is subnormal when compared with that of the control group. Values for exposed hamsters are consistently statistically different from controls at all weighing periods, 12 through 44 weeks.

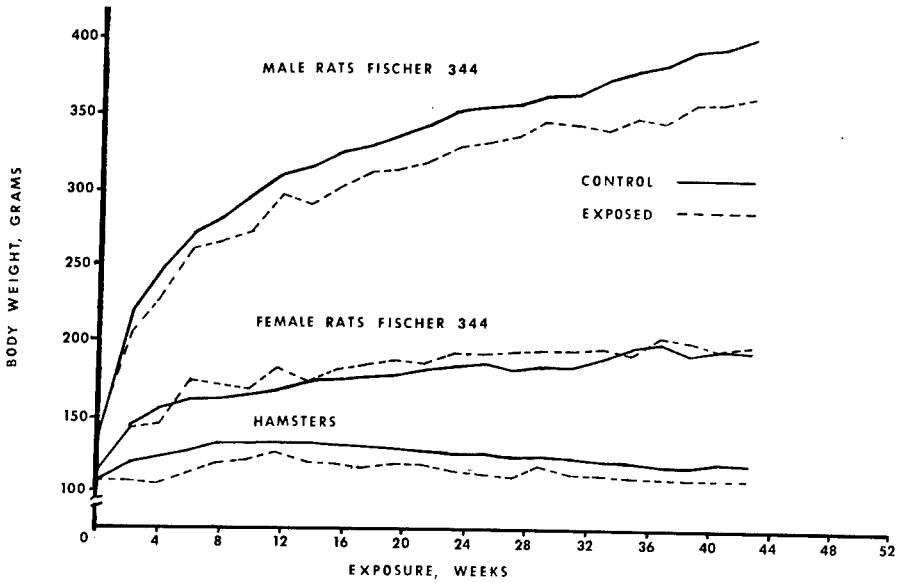


Figure 9. Effect of chronic inhalation exposure to JP-10 on growth of rodents.

Various clinical chemistry values collected for exposed dogs through 44 weeks of exposure have shown occasional statistical differences. However, they are not biologically significant and no trend to adverse hematologic effect is seen.

Firm conclusions as to the cause of death in various groups of exposed and control animals must await the results of histopathologic examination. However, mortality has been limited and absent in many cases as can be seen in Table 19.

TABLE 19. MORTALITY RATIOS FOR GROUPS OF JP-10 EXPOSED AND CONTROL ANIMALS AT 44 WEEKS OF THE STUDY.

Species, Sex	Unexposed Controls	100 ppm JP-10 Exposed
Mice, female	25/200	13/200
Rats, male	0/50	0/50
Rats, female	4/50	0/50
Hamsters, male	5/100	8/100
Dogs, male	0/4	0/4
Dogs, female	0/4	0/4

The exposures are to be completed in June 1979 and postexposure observation begun at that time. The study will continue until June 1980 when rodent necropsy will begin.

A 12-MONTH CHRONIC INHALATION EXPOSURE OF ANIMALS TO METHYLCYCLOHEXANE TO DETERMINE ITS ONCOGENIC POTENTIAL

Methylcyclohexane (MCH) is a solvent found in the aircraft fuel designated JP-9. This fuel is a mixture of three primary ingredients, JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. They also have high viscosity which causes pumping and flow problems at low temperature which are corrected by the addition of MCH to the mixture.

Chronic inhalation exposures to RJ-5 have been conducted in our laboratory and have been reported in the annual report (MacEwen and Vernot, 1975). Acute and chronic toxicity of MCH has been reported by Treon et al. (1943). Six-hour acute exposures of rabbits to inhaled concentrations of MCH above 10,000 ppm caused convulsions, light narcosis, labored breathing, salivation, and conjunctival congestion. Between 5500 ppm and 7300 ppm, lethargy and impaired coordination were the only signs.

Lazarew (1929) reported that 7500 to 10,000 ppm vapor for two hours produced narcosis in mice while 10,000 to 12,000 ppm caused death. Lehman and Flury (1943) indicated that the acute toxicity of MCH was greater than that of heptane, but less than that of octane. Similar high level narcotic effects were reported when mice were exposed to heptane vapors between 10,000 and 15,000 ppm (Fuehner, 1921). In addition, Patty and Yant (1929) reported slight dizziness in man after exposure to 1000 ppm for six minutes. Concentrations of 2000 to 5000 ppm resulted in marked vertigo, nausea, incoordination, and hilarity which persisted for several hours after exposure.

The American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH in 1976 from 500 ppm to 400 ppm or 1600 mg/m^3. The recommended short-term exposure limit (STEL) is 500 ppm or 2000 mg/m^3. These values are based on analogy to the toxicity of heptane and are identical to the TLV and STEL of heptane.

The scarcity of chronic exposure data for animals and the consequent use of analogy with other solvents for setting human exposure limits has recently been shown to be risky. Prolonged exposure to methylbutylketone and n-hexane were shown to cause peripheral polyneuropathy in man. A TLV of 500 ppm had been set for n-hexane based solely on toxicity data and comparison with other petroleum solvents such as pentane. Reports of neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1977.

These studies were undertaken to obtain the data needed to assess the safety margin of current exposure limits for methylcyclohexane.

Animal exposure concentrations of MCH for this study were selected on the basis of the current TLV (400 ppm) and 2000 ppm which appeared to be a maximum tolerated level for repeated exposure.

The exposure portion of this study began on 1 August 1978 and will continue for one year after which 20 mice, 10 rats, and 10 hamsters from each group will be necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents and dogs will be held for an additional year of observation or until the cumulative mortality in any subgroup of a species reaches 90%. Each exposure and control group of animals consists of 65 male and 65 female rats, 200 female mice, 100 male hamsters, and 8 dogs equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each sex and species which had reached the required age for tumor induction allowing for natural and toxicologic attrition.

It is necessary to use two large exposure chambers for each exposure level to house the numbers of animals selected in a manner compatible with good animal care practices. Rats and dogs are exposed in one chamber, and a companion chamber receiving the same MCH concentration houses the mice and hamsters.

The one-year inhalation exposure of animals to MCH simulates an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off. The animals have food available ad libitum during the nonexposure periods but it is removed from the chamber during exposure. Cleaning and feeding is done after the daily exposure period.

The animals used in this study are observed hourly during the one-year exposure phase and will be observed at least 6 times daily during the postexposure holding phase of the study.

Rats, hamsters, and dogs are weighed individually at biweekly intervals during exposure and will be weighed monthly during the postexposure period. Mice are weighed in groups with group mean weights followed on a monthly basis throughout the experimental period.

Blood samples are being drawn from all dogs at biweekly intervals and clinical determinations performed for a battery of tests including routine hematology tests, electrolytes, glucose, creatinine, bilirubin, serum protein, albumin, and three enzymes, SGPT, SGOT, and alkaline phosphatase. The same clinical hematology and blood chemistry tests done routinely on the

dogs will also be done on blood from the rats necropsied at the end of the one-year exposure. Organ weight data will also be compiled and evaluated for these animals.

All of the animals used in this study will be necropsied at death and a battery of approximately 33 tissues sampled for histopathology examination following the protocol used by the National Cancer Institute. Deaths in the first seven months of exposure were few and sporadic. None were attributed to MCH exposure. Although the greatest number of mouse deaths occurred in the 2000 ppm MCH exposure group, 10 of the 20 dead mice were killed by accident when an automatic water valve failed.

The generation of desired chamber concentrations of MCH is accomplished by metering liquid MCH directly into the chamber inlet air supply stream where vaporization is accomplished in sufficient air volume to prevent formation of an explosive mixture of solvent vapors. The liquid MCH is delivered from a drum using 3-5 psig air pressure with dual regulators used to prevent overpressurization. The delivery into the air supply line is metered and controlled with a glass flowmeter and 1° needle valve installed on a manifold from the storage drum and housed in an exhaust hood to prevent leakage into work areas. The stainless steel supply lines are wrapped with electrical heating tape to provide modest heat when necessary. The generation system allows 95% of the nominal chamber concentration to be achieved within 15 minutes of daily start-up of animal exposures.

Air samples are continuously drawn from the chamber during animal exposures for analysis. Each pair of chambers with the same MCH concentration is monitored by use of a total hydrocarbon analyzer which analyzes the MCH from each of the pair of chambers on a 15 minute cycle.

The exposure concentrations achieved during the first seven months of the study are shown in Table 20. Exposure concentration control has been satisfactory after the first few weeks and is reflected in the relatively small standard deviations of the means.

TABLE 20. MONTHLY MEAN METHYLCYCLOHEXANE CONCENTRATIONS MEASURED IN ANIMAL EXPOSURE CHAMBERS (PPM MCH)

Month	Chamber 1	Chamber 2	Chamber 3	Chamber 4
August	412 ± 51	396 ± 63	1878 ± 148	1847 ± 153
September	401 ± 7	398 ± 9	2023 ± 96	2001 ± 92
October	401 ± 8	399 ± 10	2050 ± 105	2021 ± 102
November	400 ± 10	399 ± ±0	2032 ± 115	1994 ± 112
December	401 ± 11	399 ± 10	2047 ± 103	2028 ± 94
January	398 ± 10	396 ± 11	2030 ± 99	1912 ± 89
February	403 ± 12	400 ± 9	1979 ± 80	1990 ± 95
March	405 ± 14	403 ± 14	2014 ± 99	2033 ± 89

Purity analyses have been made on each drum of methylcyclohexane used in the study. The drums contained 3 different lots of MCH manufactured by Eastman Organic Chemical Corporation and were designated lots A8, A9, and B8. Lot A8 contained 98.57% MCH with two impurities, 0.86% n-heptane and 0.56% toluene, while lot A9 was 98.50% pure containing 0.97% n-heptane and 0.52% toluene. Lot B8 contained 98.66% MCH, 0.74% n-heptane and 0.60% toluene. Only the two impurities were identified and the relative purity of the MCH was consistent from drum to drum within lots and between lots used in the study.

The mean body weights of MCH exposed and control rats are shown in Figure 10. Female rat weights appear to be unaffected by exposure to MCH while there appears to be a depression of growth in exposed male rats which may be dose dependent. Hamster growth for both exposure groups has been significantly depressed (Figure 11) by approximately 20%. This effect has been noticeable since the first week of exposure.

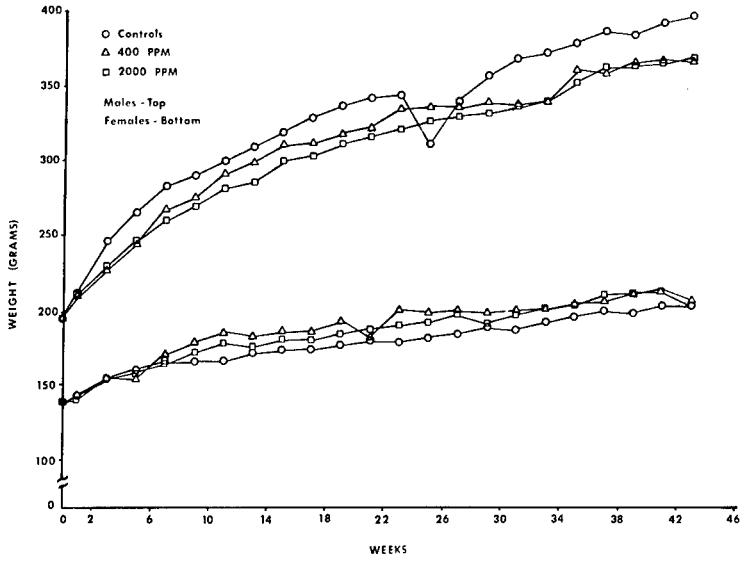


Figure 10. Mean body weights of rats during a one-year inhalation exposure to MCH.

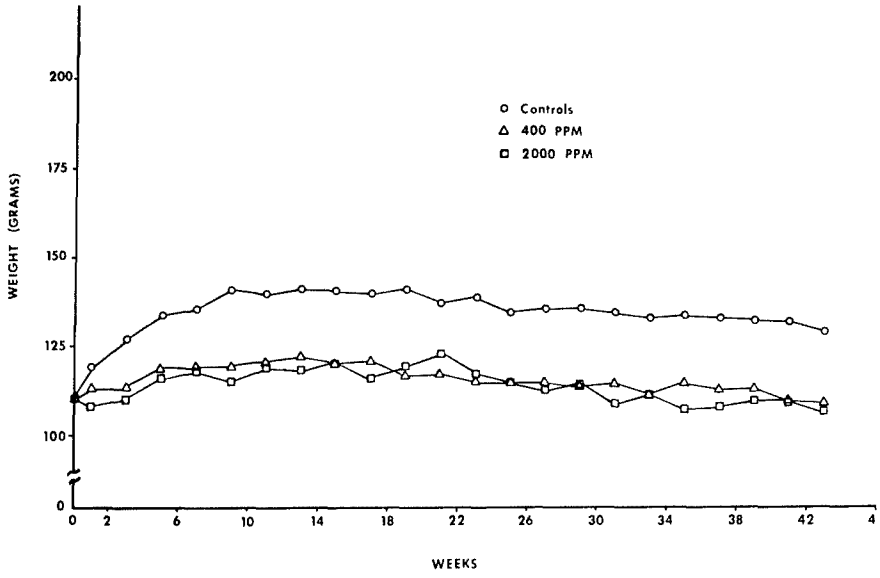


Figure 11. Mean body weights of hamsters during a one-year inhalation exposure to MCH.

Clinical determinations on samples of dog blood taken at biweekly intervals have given mostly variable but non-MCH related responses to date. The only parameter apparently affected by MCH exposure was a transient dose related increase in serum glutamic pyruvate transaminase (SGPT) levels in the first 7 weeks of exposure (Figure 12). After 29 weeks of exposure, there were no differences between exposed and control dogs. Part of the significance of the SGPT increase was due to one dog that exhibited a much greater increase than others in his exposure group with a peak value of 298 units/ml after seven weeks exposure.

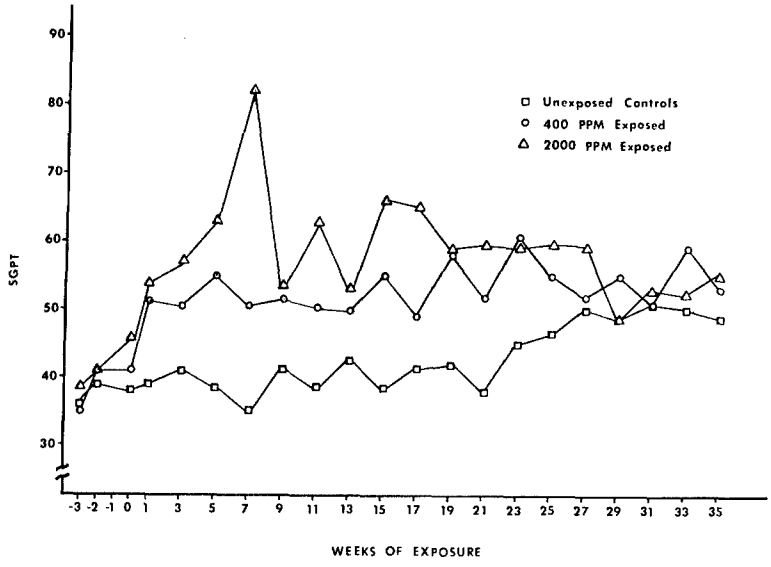


Figure 12. Effect of chronic methylcyclohexane on SGPT levels in beagle dogs.

These studies are continuing, and further information will be available at the next report period.

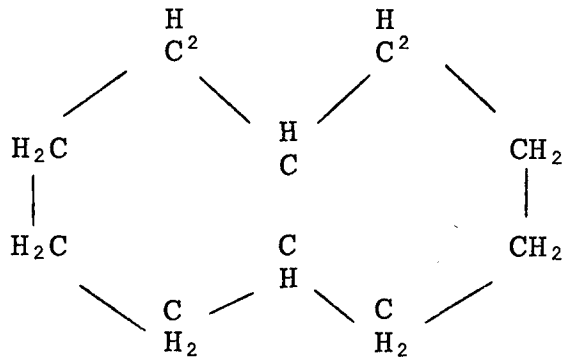
A SUBCHRONIC INHALATION TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN

Decalin (decahydronaphthalene) is an alicyclic hydrocarbon commonly used as a solvent. No threshold limit value exists for decalin, and the experimental data available are insufficient for establishing a limit. Gage (1970) described the exposure of eight rats to 200 ppm decalin for 20 days on a 6 hour/day schedule with no toxic signs and grossly normal visceral organs at necropsy. Cardini (1942) reported lung congestion, kidney and liver damage in guinea pigs exposed to 319 ppm decalin for up to 23 days.

The THRU conducted a one month inhalation exposure study of rats, mice, and guinea pigs to decalin vapors (MacEwen and Vernot, 1978). The exposures were 6 hours/day on 22 consecutive working days to 50 or 250 ppm decalin. Respiratory tract irritation was evident in the decalin exposed rats. Hydropic change in the hepatocytes and hyalin droplet formation within the proximal tubular epithelial cytoplasm were seen with increased incidence and severity in the rats exposed to decalin vapors. Mice and guinea pigs exposed to decalin also had signs of respiratory tract irritation.

Because of the incidence of the pathologic lesions noted in animals exposed to decalin vapors intermittently for one month at the THRU, it was deemed necessary that a long-term study with clinical tests and observations be developed. The present study was designed to determine the toxic effects, including oncogenesis, of a 90-day continuous exposure of test animals to decalin vapors.

Decalin is a colorless liquid with a mild characteristic odor. It is typically 99.9% pure with a cis/trans ratio of 54%/46%. Its chemical structure and physical properties are as follows:



Molecular Weight	-	138
Boiling Point Range	-	188-195 C
Flash Point	-	57 C
Specific Gravity	-	0.885-0.890

Dogs, rats, and mice were continuously exposed for 90 days to 5 ppm decalin or 50 ppm decalin in Thomas dome inhalation chambers. Groups consisted of purebred beagle dogs (3 male, 3 female) , Fischer 344 rats (75 male, 75 female), and C57Bl/6 mice (150 female). Similar groups containing the same numbers of animals were housed at the Air Force Veterinary Sciences Division (Vivarium) and served as controls. Animals had food and water ad libitum.

The experimental animals were randomized from the main group after quality control procedures and quarantine had been completed. Assignment of the animals from each species to each group was made by use of the THRU computer program RANDUM which utilizes the Fortran library subroutine RANF(X).

Upon termination of the 90-day exposure, all dogs and one-third of the rodents were sacrificed for detection of pathologic lesions caused by exposure to decalin. At 19 months postexposure, one half of the remaining rodents will be sacrificed. The animals should have attained almost a normal lifetime age at that time, and the sacrifice should give a statistically satisfactory sample of animal tissues which will not be compromised by cannibalism or postmortem degeneration. All remaining rodents will be held until the mortality in any group of the species reaches 90% of the original number. At that time, all representatives of that species will be sacrificed for gross and histopathologic examination. All animals that died during exposure or were sacrificed at 90 days were necropsied and major organ tissues taken for histopathological examination. Animals dying during the postexposure period are necropsied in accord with the NCI protocol, harvesting 33 tissues.

All animals were observed hourly during the exposure and are being observed daily during postexposure until the mortality rate becomes significant enough to warrant more frequent examination.

Rats and dogs were weighed individually at biweekly intervals during exposure and rats monthly during the postexposure period. Mice were weighed in groups and group means followed throughout the experimental period. Blood samples were drawn from dogs at biweekly intervals and from all sacrificed rats and clinical determinations done for the series of tests shown in Table 21.

TABLE 21. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND RATS EXPOSED TO DECALIN VAPORS

Hematology	Chemistry
HCT	Sodium
HGB	Potassium
RBC	Calcium
WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
	SGOT
	Bilirubin
	Creatinine
	BUN

Brain, liver, kidney, and spleen weights were obtained from the dogs and rats that were necropsied at 90 days. Bromsulphalein (BSP) retention times were measured in the dogs at the conclusion of the exposure to evaluate liver function.

Decalin vapors were generated by using the same system that was developed for a toxicity study of the jet fuel JP-5. A complete description of this system can be found in the last annual report (MacEwen and Vernot, 1978). Chamber concentrations of decalin vapors were monitored with a Beckman Model 400 hydrocarbon analyzer. The analyzed mean concentration for the entire exposure period was 5.01 ± 0.06 ppm in Chamber 1 and 50.1 ± 0.7 ppm in Chamber 2. Purity tests on the decalin used confirmed the 99.9% purity and 54/46 ratio of cis/trans isomers.

The dogs exposed to decalin vapors were generally heavier than controls throughout the exposure (Figure 13). Control dogs went through a period of slight weight loss during the first six weeks of the study.

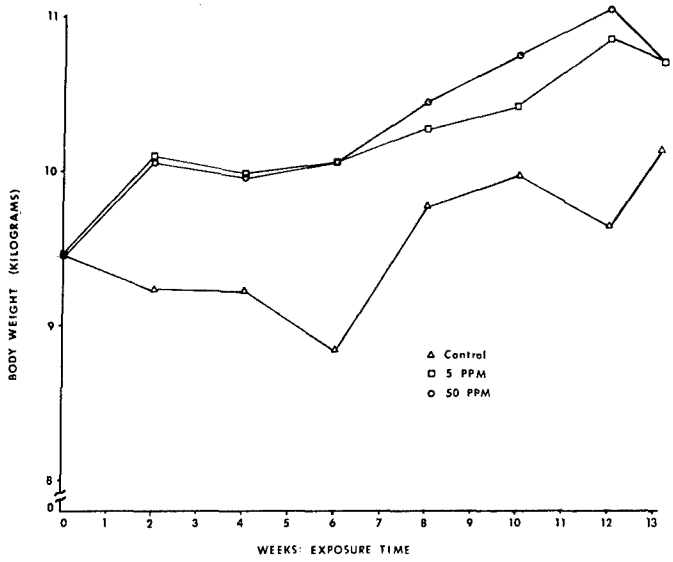


Figure 13. Effect of 90-day continuous inhalation exposure to decalin on body weight of beagle dogs.

Clinical chemistry determinations of the dog blood revealed slight but significant increases in serum globulin levels of the exposed dogs when compared to controls. These values, although increased, were within normal limits. All other blood parameters measured remained normal through the 90-day exposure to decalin vapors.

Bromsulphalein retention times measured in the dogs at the conclusion of the exposure are shown in Table 22. These results indicate that the exposure to decalin had no effect on liver function as measured by the BSP retention test.

TABLE 22. BROMSULPHALEIN RETENTION TIME IN DOGS AFTER 90 DAYS CONTINUOUS EXPOSURE TO DECALIN VAPORS

Concentration	% Retention at 10 Minutes (Mean ± S.D., N=6)
Control	14.3 ± 7.1
5 ppm	11.3 ± 4.4
50 ppm	13.5 ± 8.2

Organ weights obtained from the dogs necropsied at the conclusion of the 90-day exposure period are shown in Table 23. The liver/body weight ratios of the dogs exposed to 5 ppm decalin vapors were significantly less than controls. This is probably not an exposure related effect since the liver/body weight ratios of the dogs exposed to 50 ppm, while slightly

less than controls, were not found to be significantly different from controls. There was no measurable effect on organ weights in dogs exposed for 90 days to decalin vapors.

TABLE 23. THE EFFECT OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN ON ORGAN WEIGHT OF BEAGLE DOGS*

	Control	Decalin (5 ppm)	Decalin (50 ppm)
Body weight (kg)	10.1 ± 0.9	10.7 ± 0.9	10.7 ± 0.4
Brain weight (gm)	78.4 ± 3.6	77.7 ± 5.2	79.8 ± 3.4
Brain/100 gm body	0.78 ± 0.11	0.73 ± 0.06	0.75 ± 0.04
Liver weight (gm)	283.7 ± 32.1	269.3 ± 14.7	280.3 ± 28.6
Liver/100 gm body	2.81 ± 0.19	2.52 ± 0.15**	2.64 ± 0.31
Kidney weight (gm)	47.6 ± 5.2	46.9 ± 4.6	48.5 ± 5.0
Kidney/100 gm body	0.47 ± 0.05	0.44 ± 0.03	0.46 ± 0.06
Spleen weight (gm)	40.3 ± 24.4	38.1 ± 27.2	44.2 ± 20.3
Spleen/100 gm body	0.39 ± 0.22	0.36 ± 0.26	0.42 ± 0.19

* - Mean ± S.D., N = 6/group.
** - Significant test vs. control, $p < 0.05$

Distinct dose related lesions were not observed either grossly or microscopically in dogs exposed to decalin vapors. Of moderate significance, however, was the finding of pulmonary inflammatory lesions in both control and exposed groups. These inflammatory changes ranged from mild, focal, chronic lesions in some dogs to diffuse chronic-active bronchopneumonia in others. In most cases inflammation was attended by abundant eosinophile infiltrates. These findings suggest that many test subjects had mild verminous pneumonia caused by either migrating nematode larvae or adult Filaroides sp., lungworms.

Exposure to decalin vapors affected the body weight gains of the male Fischer 344 rats as shown in Figure 14. The differences in body weights between test and control groups were significant ($p \leq 0.01$) after 2 weeks of exposure and remain statistically significant 32 weeks postexposure. There appeared to be a mild dose related effect in that the weights of the male rats exposed to 50 ppm decalin are generally less than the male rats exposed to 5 ppm. Statistical analysis of the data failed to show a consistent significant difference between these groups, however.

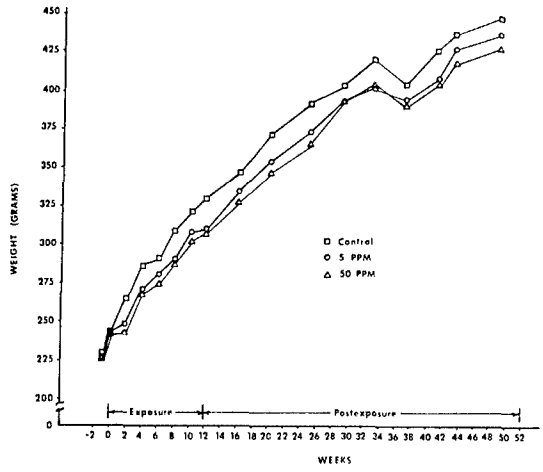


Figure 14. Growth of male Fischer 344 rats exposed 90 days continuously to decalin vapors.

Female rat weights are shown in Figure 15. Decalin exposure had only a marginal effect on the weight gains of the female rats. The group mean weight of the female rats exposed to 50 ppm decalin was significantly ($p \leq 0.01$) less than controls after 6 weeks of exposure. However, at 8 weeks of exposure, the mean weight had returned to control values. All groups of females (control included) went through a period of weight loss toward the end of the exposure period. No explanation for this weight loss has been found.

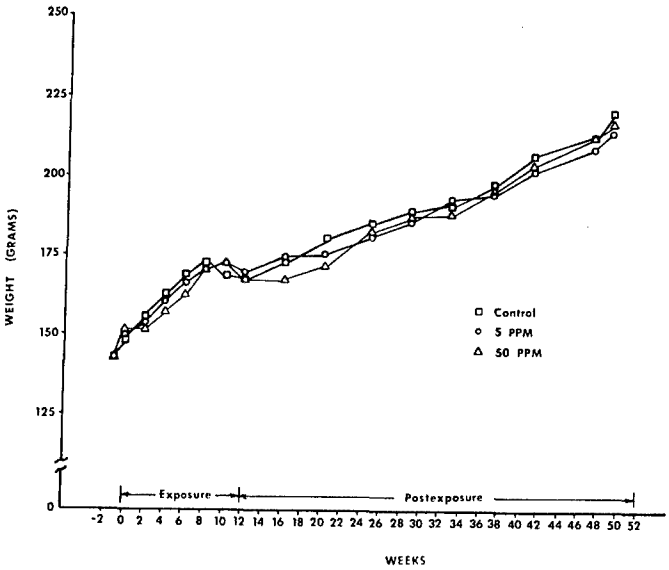


Figure 15. Growth of female Fischer 344 rats exposed 90 days continuously to decalin vapors.

Blood samples were obtained via the portal vein from all of the rats that were necropsied at the conclusion of the 90-day decalin exposure period. Over 70% of the samples obtained were hemolyzed leading to such uncertainties in the hematological and clinical chemistry results that they were rejected as nonvalid data.

Organ weights obtained from the sacrificed rats are shown in Table 24 for the males and Table 25 for the females. The increase in kidney to body weight ratios in the male Fischer 344 rats exposed to 50 ppm decalin was consistent with the increase in this parameter found in the male Sprague-Dawley rats exposed to 250 ppm decalin for 22 days on a 6 hour/day, 5 day/week basis. All other changes in organ weights can be interpreted as reflections of the changes seen in body weight.

TABLE 24. THE EFFECT OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN ON MALE FISCHER 344 RAT ORGAN WEIGHTS [a]

	Control	5 ppm	50 ppm
Body weight (gm)	327.4 ± 15.1	304.4 ± 13.6[b]	303.0 ± 14.7[b]
Liver weight (gm)	8.8 ± 0.5	7.5 ± 0.5[bd]	8.2 ± 0.7[b]
Liver/100 gm body	2.69 ± 0.13	2.47 ± 0.11[bd]	2.72 ± 0.14
Kidney weight (gm)	2.13 ± 0.33	1.92 ± 0.13[bd]	2.32 ± 0.23[c]
Kidney/100 gm body	0.65 ± 0.09	0.63 ± 0.03	0.77 ± 0.05[bd]
Spleen weight (gm)	0.56 ± 0.11	0.56 ± 0.06	0.60 ± 0.04[d]
Spleen/100 gm body	0.17 ± 0.03	0.18 ± 0.02	0.19 ± 0.11[bd]
Brain weight (gm)	1.88 ± 0.08	1.88 ± 0.07	1.90 ± 0.06
Brain/100 gm body	0.58 ± 0.03	0.62 ± 0.03[b]	0.63 ± 0.03[b]

a - Mean ± S.D., N = 25.
b - Significant test vs. control, $p < 0.01$.
c - Significant test vs. control, $p < 0.05$.
d - Significant test vs. test, $p < 0.01$.

TABLE 25. THE EFFECT OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN ON FEMALE FISCHER 344 RAT ORGAN WEIGHTS

	Control	5 ppm	50 ppm
Body weight (gm)	165.6 ± 6.7	170.6 ± 7.6[b]	169.8 ± 5.9[b]
Liver weight (gm)	4.1 ± 0.3	4.1 ± 0.3	4.3 ± 0.4[bd]
Liver/100 gm body	2.51 ± 0.13	2.40 ± 0.15[bc]	2.54 ± 0.18
Kidney weight (gm)	1.21 ± 0.07	1.22 ± 0.09	1.22 ± 0.08
Kidney/100 gm body	0.73 ± 0.05	0.71 ± 0.05	0.72 ± 0.05
Spleen weight (gm)	0.37 ± 0.05	0.38 ± 0.04	0.39 ± 0.04
Spleen/100 gm body	0.22 ± 0.03	0.22 ± 0.02	0.23 ± 0.03
Brain weight (gm)	1.73 ± 0.09	1.76 ± 0.08	1.77 ± 0.08
Brain/100 gm body	1.05 ± 0.07	1.03 ± 0.07	1.05 ± 0.07

a - Mean ± S.D., N = 25.
b - Significant test vs. control, $p < 0.05$.
c - Significant test vs. test, $p < 0.01$.
d - Significant test vs. test, $p < 0.05$.

Mild to moderate, focal renal tubular necrosis was noted at the corticomedullary junction in male rats exposed to 50 ppm decalin while similar, but minimal to mild lesions were observed in the 5 ppm exposure group. Additionally, hyaline droplets were present in the epithelial cells of the convoluted portion of the renal tubules in both groups. Neither focal tubular necrosis or hyaline droplets were noted in exposed female rats or control animals.

Mild to moderate hepatocellular cytoplasmic vacuolization was present in mice exposed to 50 ppm decalin, while minimal to mild vacuolar changes were observed in the 5 ppm exposure group. This lesion was not present in control animals. Renal lesions were not observed in mice.

This study will continue through the next year. Further information will be presented in further annual reports.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DIESEL FUEL MARINE

Diesel Fuel Marine (DFM) is the standard fuel used by a large number of ships in the U.S. Naval fleet. The DFM used in this study was derived from traditional petroleum sources and is typically a mixture of aliphatic hydrocarbon compounds with a small quantity of aromatic compounds.

A detailed discussion of the protocol, contaminant generation and monitoring system and available results at the conclusion of the 90-day exposure were presented in the last annual report (MacEwen and Vernot, 1978). The present report will update and summarize the results of ths study which were not available for the previous annual report.

Male and female beagle dogs, male and female Fischer 344 rats, and female C57Bl/6 mice were continuously exposed to concentrations of 50 mg/m^3 or 300 mg/m^3 DFM vapors for 90 days in Thomas dome inhalation chambers. Unexposed controls were held in a separate facility. At the conclusion of the exposures, all dogs and 1/3 of the rodents were sacrificed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to DFM.

The remaining rodents are presently being held for postexposure observation for 19 months. At that time, 1/2 of the remaining rodents will be sacrificed.

Rats are weighed individually on a monthly basis during postexposure holding while the mice are weighed in groups.

There was a significant effect on bodyweight gains in male Fischer 344 rats exposed to DFM vapors for 90 days as shown in Figure 16. The mean body weights of both DFM exposed groups are significantly ($p \leq 0.01$) less than unexposed controls through eleven months of the postexposure observation period. A dose related effect is evident.

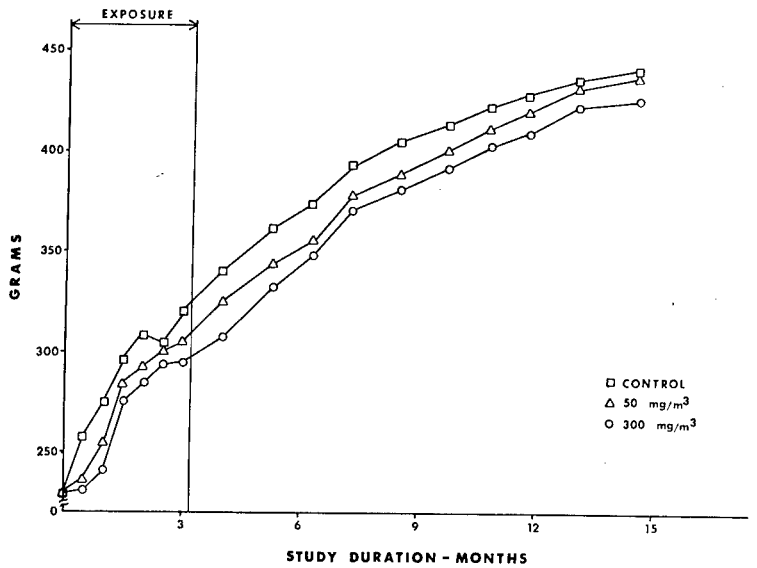


Figure 16. Effect of 90-day continuous inhalation exposure on male rat body weight.

The body weights of the females are shown in Figure 17. Female rats exposed to 300 mg/m^3 DFM had a slight weight loss after 8 weeks of exposure. The body weights in this group became significantly ($p \leq 0.01$) less than controls at that time and continue to be less than controls through the 14th month of the study. The body weights of the female rats exposed to 50 mg/m^3 DFM vapors did not differ from controls until the seventh month of the study when a significant ($p < 0.01$) difference between this test group and its controls was noted.

Acute and chronic inflammatory processes were seen in the nasal mucosa in approximately 10% of both groups of rats after 90 days of exposure. No such lesions were found in unexposed control rats. Lymphoid hyperplasia of the bronchial submucosa was seen in 39% of the controls, 62% of the 50 mg/m^3 exposure group and 54% of the 300 mg/m^3 exposure group. This lesion is nonspecific and usually denotes chronic irritation. It is seen as an early lesion of chronic respiratory disease in rats.

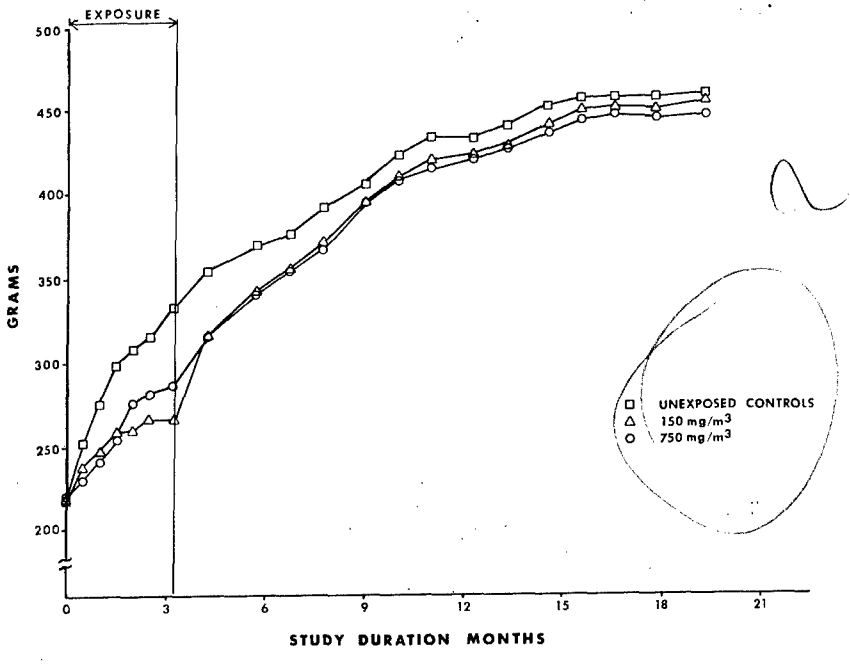


Figure 17. Effect of 90-day continuous inhalation exposure on female rat body weight.

Hyaline degeneration of the cytoplasm of the convoluted renal tubules was seen in 68% of the male rats exposed to 50 mg/m^3 DFM vapors and in 84% of the male rats exposed to 300 mg/m^3 DFM vapors. This type of lesion was not found in unexposed control males or in any of the female rats. This lesion is considered a mild reversible degenerative process that results from accumulation of protein in the cytoplasm of convoluted tubule cells due to an incapacity of the cells to transfer resorbed blood protein from the glomerular filtrate back into the blood. An associated lesion (nephropathy) was seen in 96% of the male rats exposed to 300 mg/m^3 DFM. Female rats in this group as well as all the rats exposed to 50 mg/m^3 DFM, and unexposed controls were free of this change. The nephropathy is described as the presence of granular casts within the viable collecting tubules localized at the corticomedullary junction of the kidney. The granular casts were probably cell detritus resulting from increased cell turnover among degenerative cells in the convoluted segment of each affected nephron which accumulated in the collecting tubules. The nephropathy was seen in connection with a variable and mild chronic interstitial nephritis.

There were no marked or startling histopathologic changes seen in the mice exposed to DFM vapors. The only lesions seen which occurred in more than a few animals and were deemed dose dependent were acute focal inflammation of the liver and splenic hemosiderosis. The liver lesions were mild, widely scattered microscopic multifocal accumulations of chronic inflammatory cells seen in association with death and/or hyalinization of liver cord cells. These lesions involved only a few hepatocytes and were not seen in association with any other manifestations of liver disease. The hemosiderin content of the spleen was moderately increased in the mice exposed to 300 mg/m^3 DFM vapors.

The postexposure observation period of this study continues. The sacrifice at 19 months postexposure is scheduled for the fall of 1979. Information from this sacrifice will be discussed in future annual reports.

EVALUATION OF THE TOXIC EFFECTS OF A 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5 JET FUEL

The use of jet engines in military and commercial aircraft has led to the use of a number of petroleum distillate fuels with special properties. These fuels are generally less volatile than gasoline fractions used in conventional internal combution engines.

The THRU has conducted studies on a number of jet fuels to determine the toxic effects of prolonged inhalation of the fuel vapors. In 1977, a 90-day continuous inhalation exposure of animals to the jet fuel JP-5 was conducted. The study was conducted at the request of the U.S. Navy and was designed to determine the toxic effects as well as oncogenic potential of JP-5. Conditions were chosen for the experiment to simulate exposure conditions peculiar to the Navy and to permit comparison with previous exposures to petroleum distillate fuels and future exposures to fuels derived from shale oil sources.

A detailed discussion of the protocol, contaminant generation and monitoring system, and available results at the conclusion of the 90-day exposure were presented in the last annual report (MacEwen and Vernot, 1978). The present report will update and summarize the results of the study which were not available for the previous annual report.

Male and female beagle dogs, male and female Fischer 344 rats, and female C57Bl/6 mice were continuously exposed to concentrations of 150 mg/m^3 or 750 mg/m^3 JP-5 vapors for 90 days in Thomas dome inhalation chambers. Unexposed controls were held in a separate facility. At the conclusion of the exposures, all dogs and 1/3 of the rodents were sacrificed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to JP-5.

The remaining rodents are being held for postexposure observation for 19 months. At that time, 1/2 of the remaining rodents will be sacrificed.

There was a significant effect on the body weight gains of the male Fischer 344 rats exposed to JP-5 vapors for 90 days as shown in Figure 18. The body weights of the male rats exposed to 750 mg/m^3 JP-5 vapors remain significantly ($p \leq 0.01$) less than unexposed controls through the 19th month of the study. The growth of the male rats exposed to 150 mg/m^3 JP-5 was significantly ($p \leq 0.01$) lower than unexposed controls through the 12th month of the study. However, the body weights of this exposure group increased to nearly the weight of control animals and were not significantly different from the controls at the 16th month of the study. There has been no effect on the weight of female rats exposed to JP-5 vapors for 90 days as shown in Figure 19.

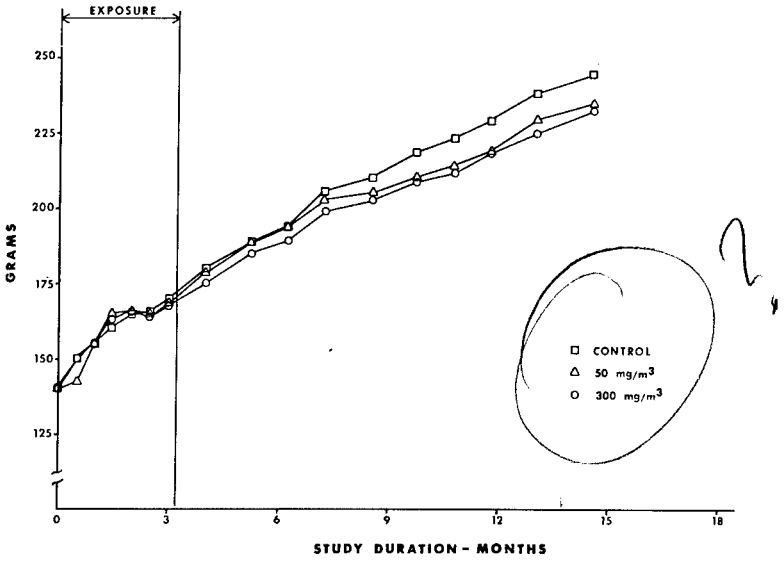


Figure 18. Effect of 90-day continuous inhalation exposure to petroleum JP-5 on male rat body weight.

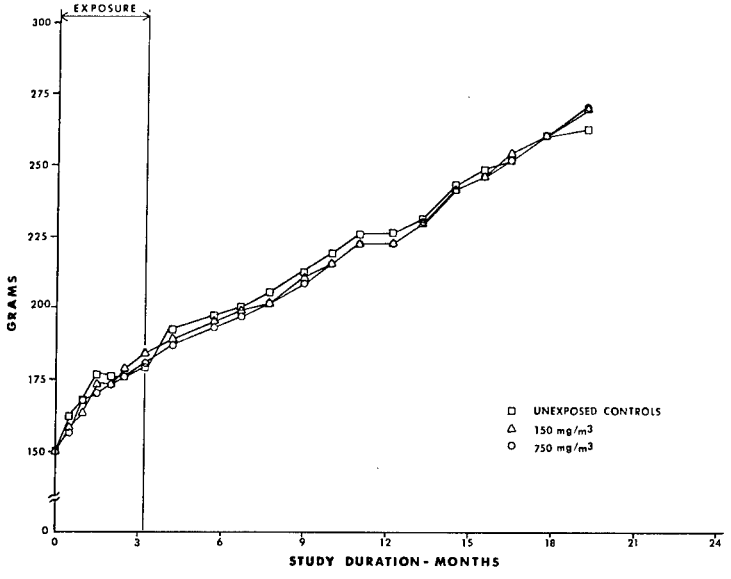


Figure 19. Effect of 90-day continuous inhalation exposure to petroleum JP-5 on female rat body weight.

The postexposure observation period of this study continues. The sacrifice at 19 months postexposure is scheduled to occur in June of 1979. Information from this sacrifice will be discussed in future annual reports.

EMERGENCY EXPOSURE LIMITS

Studies were conducted during the past year to develop information necessary for setting Emergency Exposure Limits (EEL's) for JP-10 and methylcyclohexane (MCH). EEL's are defined by Frawley (1964) as "concentrations of contaminants that can be tolerated without adversely affecting health but not necessarily without acute discomfort or other evidence of irritation or intoxication. They are intended to give guidance in the management of single, brief exposures to air-borne contaminants in the working environment." Limits of this type are not intended for maintenance of exposures in a working environment but for engineering guidance and planning for storage limits and emergency preparedness. The levels selected must not produce sensory irritation or impairment of judgment that might interfere with self rescue.

EMERGENCY EXPOSURE LIMIT STUDIES WITH JP-10

JP-10 is a synthetic saturated polycyclic hydrocarbon which, because of its high density and other physical chemical properties, is utilized as a jet fuel either alone or as a major constitutent (70%) of JP-9 fuel. In the latter application, it has been substituted for RJ-4, a reduced dimer of methylcyclopentadiene. Since no information has appeared in the literature concerning the toxicological properties of JP-10, a series of acute studies were done to characterize them and provide background information.

Acute inhalation studies with female mice, male and female rats, and male hamsters showed mice to be the most susceptible species. Essentially saturated vapors for two hours resulted in death of all exposed mice and female rats. Five of six male rats died after two hours exposure while no hamsters succumbed following four hours of exposure.

Four-hour LC_{50} exposures were performed on male and female rats giving the following results:

Male rats = 1221 (1174-1259) ppm
Female rats = 1194 (1104-1287) ppm.

An exact LC_{50} was not obtained for mice as the difference between zero and 100% mortality was so slight that an attempt to achieve partial mortalities was not made. A concentration of 900 ppm resulted in no deaths while 955 ppm caused complete mortality in groups of rats.

Symptoms in these exposures were eye irritation, fine tremors, prostration, followed by convulsions and death. Survivors of high level exposures showed hind quarter paralysis which lasted throughout the 14-day observation period or to time of death, whichever occurred first.

The purpose of this experiment is to establish emergency exposure limits for time periods of 60, 30, and 10 minutes. An emergency exposure limit is defined as that concentration which will not cause chronic or irreversible tissue damage nor produce CNS effects which could impair coordination and prevent a man from self rescue.

JP-10 is a single chemical entity chemically identified as tricyclo($5.2.1.0^{2,6}$) decane. Gas chromatographic analysis of samples from two drums of this material indicated that it is 98% pure JP-10 and 2% miscellaneous unidentified impurities. The material was supplied by the Air Force and was received from Suntech, Inc., Marcus Hook, Pennsylvania.

The known physical properties of JP-10 are as follows:

Molecular weight	-	136
Boiling point	-	360 F
Density, 70 F	-	0.940
Viscosity, 70 F	-	3.5 CP
Flash point	-	135 F.

Groups consisting of 20 male Sprague-Dawley rats and 20 female ICR mice were exposed to JP-10 vapors for one-hour periods. A control group of each species was maintained for comparison to the test group. Ten animals of each species, including controls, were killed immediately following exposure for examination while the remaining animals were observed for 28 days postexposure.

Once the concentration causing no observable effect in rodents was achieved, four dogs were also exposed at that level. A group of four control dogs was maintained for comparative purposes.

Rodent exposures were conducted in a small Rochester chamber while the dogs were exposed in a larger chamber. The rats and mice were group housed in large cages (24" x 24") during exposure to allow for greater freedom of movement and, thus, provide more opportunity for visual observations. Dogs were not confined within the exposure chamber.

The animals used in this experiment were male, Sprague-Dawley rats and female ICR mice, both supplied by Harlan Industries of Indianapolis, Indiana. The purebred beagle dogs wereprovided by the Air Force. The rodents were fed ad libitum and received a cage change twice per week. The dogs were fed once per day and had their cages cleaned daily.

JP-10 vapors were produced by generating a known liquid flow thrugh a spiral glass evaporator. A measured airflow through the evaporator carried the vapors into the Rochester chamber. An airflow of a minimum of 10 cfm was maintained in the exposure chamber.

The chamber concentration was measured using a hydrocarbon analyzer which was calibrated using 100 liter Mylar® bags containing known standard concentrations of the contaminant.

All animals were carefully observed for signs of toxic stress during and after the exposure period. The rats were weighed immediately prior to exposure on the first, second, and third days postexposure and weekly thereafter.

Prior to exposure, the beagle dogs were trained to perform four basic tasks. The dogs were trained to fetch, come, stay, and lead. It was necessary to spend considerable time acclimating the dogs to the laboratory environment. Approximately six weeks of training was done. The dogs were tested weekly prior to the experimental exposure and twice immediately after exposure.

Besides the field trial evaluation, the dogs were neurologically examined after exposure using the following tests: flexor reflex, extensor thrust reflex, tonic neck reflex, tonic eye reflex, righting reflex, and placing reflex. Each reflex was tested by the method described by Hoerlein (1971).

The dogs were weighed before exposure and at 2 and 4 weeks postexposure. On the same schedule, blood samples were taken on each dog for the following determinations:

HCT	SGPT	Sodium
HGB	SGOT	Potassium
RBC	Alkaline Phosphatase	Glucose
WBC	Bilirubin	BUN.

Gross and histopathologic examinations were done on all experimental and control animals from this study. All major organs were examined and sampled with special emphasis on the liver and kidney.

The first one-hour concentration tested, 150 ppm to both rats and mice, caused immediate hyperactivity in both species. Coordination of both species remained normal throughout the exposure.

The second one-hour exposure, to a mean concentration of 254 ppm, also caused hyperactivity in both animal species. After 51 minutes of exposure, one mouse had a tonoclonic spasm which lasted for 20 seconds, after which it appeared relatively normal. The convulsion of this animal would negate this concentration for consideration of an emergency exposure level.

No signs of stress were noted during the subsequent 28-day observation period in either species, and mean body weight gains of the test animals compared favorably with their respective controls. Gross pathologic examinations of the animals which were sacrificed immediately following exposure and those sacrificed after 28 days revealed no lesions which could be attributed to the JP-10 exposure.

From the above rodent data, it appeared that a concentration of approximately 150 ppm was a safe one-hour exposure limit for rats and mice. The dog exposure was then designed for a nominal concentration of 150 ppm wherein the dogs would be carefully observed during exposure and tested post-exposure for neurological effects.

The four test dogs were exposed to a mean measured concentration of 151 ppm for one hour. The dogs behaved normally throughout the exposure showing no signs of irritation or CNS effects. Immediately following the exposure, each dog performed the four trained tasks with its assigned animal trainer. All dogs performed this exercise adequately to the standard established during the training program. The subsequent neurological testing of each dog revealed no exposure-related effects. During the 28-day postexposure observation period, all dogs appeared normal. Blood samples examined at 14 and 28 days postexposure showed all normal values. Gross pathology at necropsy revealed no exposure related lesions.

In a similar manner, two concentrations were selected for testing at the 30-minute time period. The first exposure was to a mean concentration of 823 ppm. This resulted in the usual increase of activity in both species, as well as tonoclonic spasms in several mice starting at 16 minutes and continuing to the conclusion of the 30-minute exposure. Two

mice died after being removed from the exposure chamber. The rats showed no adverse symptoms during or following exposure.

Following the 28-day observation period, mean body weight gains of the test rats and mice did not differ significantly from their respective control group. Gross pathology examination revealed no lesions related to treatment.

The second rodent exposure for this time period was done at 723 ppm. The only toxic sign noted was increased activity of both species for the duration of the exposure. The 28-day observation period for these animals was uneventful with mean weight gains and gross pathology within normal parameters.

To complete this phase of the study, four dogs were exposed to a mean concentration of 718 ppm JP-10 for 30 minutes. The vapors caused slight lacrimation in the dogs after six minutes of exposure. The lacrimation did not increase in severity during the remaining 24 minutes of exposure. One dog had a violent coughing spell after 25 minutes of exposure which continued, off and on, for approximately three minutes. During the final two minutes of exposure, the dog appeared normal.

Immediately following exposure, the dogs performed their learned tasks in a routine manner, and reflexes were normal. Blood parameters tested at 14 and 28 days postexposure were within normal limits.

Because of an unavoidable delay in receiving additional rats, mice were exposed alone to the following 10-minute exposures. The first exposure, to a mean concentration of 1218 ppm, caused an increase in activity as seen in the previous exposures. No other observable symptoms were noted throughout the remaining exposure time or during the subsequent 28-day observation period.

The second exposure was to a mean concentration of 1311 ppm. During the 10-minute exposure period, the only sign noted was the usual increased activity of the mice. However, upon removal, one mouse had fine tremors and poor coordination while several other mice showed eye irritation and slight loss of coordination.

Gross pathology of the mice sacrificed immediately following exposure and after 28 days did not show any lesions which could be attributed to the chemical insult. Mean weight gains of the test mice after 28 days compare favorably with the mean weight gains of the control mice.

Rat exposures for a 10-minute time period are still to be conducted and will be reported at a later date. Rather than hold up the dog experiment until the rats were completed, it was decided that a concentration for the dog exposure would be based on information obtained in the mouse exposures.

The trained dogs were exposed to a mean concentration of 1000 ppm for 10 minutes. By two minutes, all dogs showed signs of eye irritation although only one dog experienced lacrimation. Two dogs displayed fine tremors at the conclusion of exposure. In general, all dogs appeared less active during exposure compared to the two previous dog exposures.

Following exposure, all dogs performed the learned tasks with their trainers. Reflexes, tested by the Air Force veterinarians, appeared to be normal. Blood parameters examined at 14 and 28 days were all within normal limits.

Histopathology results are not yet available on the animals used in this study. Since the results of the histopathologic examinations will play a very important part in determining irreversible damage to tissues, it would be presumptuous to draw any conclusions at this time. However, if there are no histopathology findings of a significant nature, the concentrations used for the exposure of each time limit would be the recommended short-term exposure limit. These times and concentrations are 150 ppm for 60 minutes, 600 ppm for 30 minutes, and 1000 ppm for 10 minutes. The selection of 600 ppm at 30 minutes is based on the slight lacrimation of the dogs eyes and mild cough experienced at 700 ppm. We believe a concentration of 600 ppm would eliminate this problem.

METHYLCYCLOHEXANE ONE-HOUR EMERGENCY EXPOSURE LIMIT STUDIES

Methylcyclohexane (MCH)is a solvent found in the aircraft fuel designated JP-9. This fuel is a mixture of three primary ingredients: JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. They also have a higher viscosity causing flow problems at low temperatures which is the reason for the addition of MCH to the mixture.

Chronic inhalation exposures to RJ-5 have been conducted in our laboratory and are reported in the annual report (MacEwen and Vernot, 1975). Acute and chronic toxicity studies on MCH have been reported by Treon et al. (1943). Six-hour, acute exposures of rabbits to inhaled concentrations of MCH above

10,000 ppm caused convulsions, light narcosis, labored breathing, salivation, and conjunctival congestion. Between 5500 and 7300 ppm, lethargy and impaired coordination were the only signs.

Lazarew (1929) reported that 7500 to 10,000 ppm for two hours produced narcosis in mice while 10,000 to 12,500 ppm caused death. Lehman and Flury (1943) indicated that the acute toxicity of MCH was greater than that of heptane, but less than that of octane. No human toxicity information exists for MCH.

The American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH in 1976 from 500 ppm to 400 ppm or 1600 mg/m^3. The recommended short-term exposure limit (STEL) is 500 ppm or 2000 mg/m^3. These values are based on analogy of the toxicity of heptane and are identical to the TLV and STEL of heptane.

It is felt that even though the narcotic effects of MCH and heptane on mice are similar at very high concentrations, the low level CNS effects may not be the same. It is the purpose of this study to find a concentration of MCH that will not cause chronic or irreversible tissue damage nor produce CNS effects which could impair coordination or prevent a man from self rescue.

The MCH used in this study was a colorless liquid produced by Eastman Organic Chemicals, Rochester, New York. The pertinent physical properties of the reagent grade MCH are as follows:

Molecular weight	- 98.18
Boiling point @ 760 mm	- 100.93 C
Density 25°/4°	- 0.76501
Lower explosive limit (% by volume in air)	- 1.15
Flash point (closed cup)	- 25 F
Vapor pressure	- 43 mm

Air saturated with methylcyclohexane at 20 C and 760 mm contains 56,500 ppm vapor. At these same conditions, 1 ppm vapor = 0.00401 mg/ℓ and 1 mg/ℓ = 249.5 ppm.

Groups consisting of 20 male Sprague-Dawley rats and 20 female ICR mice were exposed to MCH vapors for one-hour periods. A control group of each species was maintained for comparison to the test group. Ten animals of each species, including controls, were sacrificed immediately following exposure while the remaining animals were observed for 28 days postexposure.

Once the concentration causing no narcotic effect in rodents was achieved, four dogs were exposed at that level. A group of four control dogs were also maintained for comparative purposes.

The exposures were conducted in standard inhalation chambers. The dogs were unconfined during exposure within the chamber while rodents were group housed in large cages to provide more opportunity for visual observation.

The animals used in this experiment were male Sprague-Dawley rats and female ICR mice, both supplied by Harlan Industries of Indianapolis, Indiana. The purebred beagle dogs were provided by the Air Force. The rodents were fed ad libitum and received a cage change twice per week. The dogs were fed once per day and had their cages cleaned daily.

MCH vapors were produced by generating a known liquid flow through a spiral glass evaporator. A measured airflow through the evaporator carried the vapors into a Rochester chamber where an airflow of a minimum of 30 cfm was maintained.

The chamber concentration was measured using a Miran I infrared analyzer. Calibration of the Miran I was done using 50 liter Mylar® bags containing known concentrations of the contaminant.

All animals were carefully observed for signs of toxic stress during and after the exposure period. The rats were weighed immediately prior to exposure and at 1, 2, 3, 7, 14, 21, and 28 days postexposure.

Prior to exposure, the beagle dogs were trained to perform four basic tasks. The dogs were trained to fetch, come, stay, and lead. It was necessary to spend considerable time acclimating the dogs to the laboratory environment. Approximately six weeks of training was done including weekly tests prior to the experimental exposure and twice immediately after exposure.

In addition to the field trial evaluation, the dogs were neurologically examined after exposure using the following tests: flexor reflex, extensor thrust reflex, tonic neck reflex, tonic eye reflex, righting reflex and placing reflex. Each reflex was tested by the method described by Hoerlein (1971).

The dogs were weighed before exposure and at 2 and 4 weeks postexposure. On the same schedule, blood samples were taken on each dog for the following determinations:

HCT	SGPT	Sodium
HGB	SGOT	Potassium
RBC	Alkaline Phosphatase	Glucose
WBC	Bilirubin	BUN.

Gross examinations were done on all experimental and control animals while histopathology was done on half of the animals from this study. All major organs were examined and sampled with special emphasis on the liver and kidney.

The first concentration tested, 6564 ppm to rats and mice, caused immediate hyperactivity in both species. A slight loss of coordination was first seen in mice at 12 minutes followed by rats at 29 minutes. Prostration was noted in both mice and rats at 42 and 54 minutes, respectively. At 55 minutes, one mouse experienced tonoclonic spasms which lasted for 15 to 20 seconds. No deaths resulted from this one-hour exposure.

No signs of stress were noted during the subsequent 28-day observation period in either species, and mean body weight gains of the test animals compared favorably with their respective controls. Gross pathology examinations of the animals that were sacrificed immediately following exposure and those sacrificed after a 28-day observation period revealed no lesions which could be attributed to the MCH exposure.

The second concentration tested was to rats alone. This exposure, to a mean concentration of 4172 ppm, caused increased activity in the animals for the duration of the experiment. The activity demonstrated in this exposure was less than that seen during the 6564 ppm exposure, and the rats maintained normal coordination throughout with no observable CNS effects.

The mean body weight gain of the exposed rat group was slightly less than the control group but well within the normal weight gain range which would be expected for this time period. Gross pathology examination of these rat groups failed to show any contaminant-related lesions.

An equivalent mouse exposure was run at a mean concentration of 4758 ppm. The only observable sign noted was hyperactivity which lasted during the extent of the exposure period. Following exposure, the mice returned to their normal activity pattern. The effect of this contaminant level on the mice

appears to be very similar to the effect reported for rats at 4172 ppm. No loss of coordination or CNS effects were noted during or after the one-hour exposure.

Mean body weights of the test mice at 28 days did not differ significantly from the controls. Gross pathology examination of the animals, both immediately after exposure and at 28-days postexposure failed to reveal any exposure related lesions.

From the above rodent data, it appeared that a concentration of approximately 4200 ppm was a safe one-hour exposure limit for rats and mice. The dog exposure was then designed for a nominal concentration of 4200 ppm wherein the dogs would be observed carefully during exposure and tested postexposure for neurological effects.

The four test dogs were exposed to a measured mean concentration of 4071 ppm for one hour. The dogs all acted normally throughout the exposure showing no signs of eye or nose irritation or effects upon coordination.

Immediately following the exposure, each of the dogs performed trained tasks with its assigned trainer. All dogs performed this exercise adequately to the standard established during the training program. The subsequent neurological testing of each dog revealed no exposure related effect. During the 28-day postexposure observation period, all dogs appeared normal.

Blood samples examined at 14 and 28 days postexposure showed all normal values. Gross pathology at necropsy revealed no exposure related lesions.

Histopathologic examination of the test and control dogs showed no significant lesions. Examinations were also done on one-half the control and test rodents from the low level exposures. During this examination, two test rats that had been held twenty-eight days postexposure had convoluted tubule adenomas of the kidneys. It is unusual to find lesions of this kind in rats of this age (73 days), and it is unlikely that this lesion would develop in this short time span after a single exposure to the compound. However, this lesion was not seen in the rats sacrificed immediately after exposure or in either of the two control rat groups.

To determine whether this lesion was real or an anomaly, the kidneys from the 6700 ppm group were examined. Three sections were examined from the kidneys of each of the ten test rats. No adenomas or abnormal lesions were found in the kidneys of these rats. Therefore, it appears that the two adenomas seen in the low level rats were anomalies and had nothing to do with exposure effects.

The only change noted in mice exposed to the lowest level of MCH was a minimal to mild reversible cytoplasmic change in the liver. This lesion was seen in five of the test mice and in one control mouse.

The MCH concentrations selected for the dog and rodent exposures (4071 to 4758 ppm) caused no adverse signs of toxic stress during or after exposure, and the animals gained weight normally during the subsequent 28 days. Dog blood parameters remained normal throughout the 28-day observation period. Pathologic findings in the exposed animals were essentially the same as that seen in the respective control animals.

The MCH concentrations are in the same range as heptane concentrations reported by Patty and Yant (1929) which caused considerable narcosis in man. Although there are no human MCH data for comparison, our experiments indicate that the effects of these two compounds, while reported to be similar in animals at concentrations above 10,000 ppm, are not necessarily similar at the 4000 ppm range.

While man is reported to lose coordination and experience marked vertigo and nausea after six minutes of exposure to this concentration of heptane, the dogs challenged with MCH remained alert and coordinated throughout a one-hour exposure period. At no time during the postexposure trials did the dogs show any signs of CNS effects. During the retrieving portion of the dog trials, the dogs ran rapidly while chasing a ball and showed normal coordination in the process.

Until human MCH exposure data are available which would show that man is more susceptible to the vapors of MCH than the animals tested, one could assume that the responses would be similar. Based on the effects seen in the mice, rats, and dogs, there is no reason to believe that a concentration of 4000 ppm of MCH would hamper self rescue or cause any irreversible damage to a man within a sixty-minute period. This recommendation of 4000 ppm for a one-hour emergency exposure limit is based solely on animal data, and any human exposure information to the contrary would preclude this recommendation. The same EEL limit set for the one-hour exposure to MCH should also apply for shorter time periods since levels as low as 6554 ppm produced incoordination and spasms in rodents.

ACUTE TOXICITY STUDIES OF AIR FORCE AND NAVY MATERIALS

Several compounds were submitted to the Toxic Hazards Research Unit for acute toxicity screening tests to determine the potential hazard of these materials in a manner that could be compared with other candidate materials. The specific toxicity tests conducted on each material were related to their use and physical characteristics but not all tests were conducted on every material submitted by the participating agencies.

NAVY MATERIALS

Determination of the Acute Toxicity Potential of Navy Antifouling Paint Formulations

Cuprous oxide is one of the biocidal chemicals being used in various paint formulations for painting the exterior surfaces of ships. This compound is an effective antifouling agent against organisms such as barnacles, tubeworms, algae, sponges, etc.

Previous tests on this material indicated a low order of toxicity (LD_{50} greater than 5 gm/kg) when administered orally. Application of the compound to intact and abraded skin produced no irritation. The acute dermal toxicity was found to be greater than 20 gm/kg while instillation of the compound into rabbit eyes caused positive eye irritation. The material sprayed for one hour into a chamber containing rats caused no mortality or any observable effects in the rats at necropsy.

Acute toxicity information is needed on five antifouling paint formulations which are to be submitted to the Environmental Protection Agency for Pesticide Product Registration. In addition to the five formulations, the technical material (cuprous oxide) found in four of the paint formulations is also being reexamined for acute toxic properties.

The acute tests being attempted on each material are as follows:

a. Oral LD_{50}
b. Dermal LD_{50}
c. Primary skin irritation
d. Primary eye irritation
e. One-hour inhalation LC_{50}.

All test material has been supplied by the Navy. The six materials being tested are listed below:

1. Paint formulation 105
2. Paint formulation 121/63
3. Paint formulation 129/63
4. Paint formulation 1020A
5. Paint formulation 134
6. Cuprous oxide

Oral Toxicity LD_{50} - Rats

When possible, the compounds were administered undiluted. When this could not be done, a suitable vehicle was selected.

Syringes and oral dosing needles were used to administer the compounds to the rats. The animals were fasted for at least 16 hours prior to administration of the oral dose. This allowed for more uniform absorption since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition. The injection volume for the rats was 0.01 ml/gm which would result in the average rat receiving a volume of 2.5 ml. The rats were weighed individually at the time of dosing to determine the proper dosage volume.

Initial dose level for each compound was 20 ml/kg. If no deaths resulted from this dose, no further dosing was done. If deaths occurred, lower concentrations were selected in order to achieve an LD_{50}. Five rats were dosed at each level and the LD_{50} with its 95% confidence limits calculated using the moving average interpolation method of Weil (1952).

Deaths that occurred during the 14 days following the administration of the single dose were included in the final mortality tally. Any animals that survived the 14-day post-exposure period were weighed and sacrificed at that time.

Primary Skin Irritation - Rabbits

Primary irritation to the skin was measured by a patch-test technique on the abraded and intact dorsal skin area of the albino rabbit, clipped free of hair 24 hours prior to application. Allowing 24 hours to elapse after clipping permits any abrasions resulting from the clipping to heal. Six rabbits were used for each compound.

The test material was applied undiluted and the quantity was 0.5 ml. The application site was covered with a 1 x 1 inch piece of surgical gauze two layers thick. Each patch was secured with two pieces of 1-inch adhesive tape. The entire area was then covered with dental dam secured by tape. The rabbits were fitted with leather restraining collars to prevent disturbance of the patch area.

At the end of the 24-hour application period, the collars, dental dam, and patches were removed. Any residual test material was removed by gentle sponging with a towel. Any reactions resulting from the test material were evaluated using the scoring method of Draize et al. (1959). Readings were made again at 72 hours (48 hours after the first reading). An equal number of exposures were done on intact and abraded skin. The abrasions are minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding.

Acute Eye Irritation - Rabbits

One-tenth milliliter of the undiluted sample was applied to one eye of each of six albino rabbits. The opposite eyes were untreated and served as controls. Examinations for gross signs of eye irritation were made at 24, 48, and 72 hours following application. Scoring of irritative effects was done according to the method of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately. In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score. Cornea and iris scores are heavily weighed because of the essential role of these organs in vision.

Acute Dermal Toxicity - Rabbits

Male albino New Zealand rabbits weighing approximately 5 lbs. were used as the experimental animals. All rabbits were clipped as closely as possible with an Oster clipper having surgical blades and a vacuum attachment to prevent the fur from flying around the laboratory. The back of the rabbits and the sides down to about half way to the stomach area were clipped from the saddle area to the shoulders to the top of the rear leg area.

The animals were individually weighed prior to dosing to determine the proper dose volume. The proper volume of the liquid material was applied undiluted to the back of the rabbit and was divided as equally as possible between the two sides of the back. The dose was kept in place by applying 8 ply gauze patches over the liquid on each side of the back. A patch of latex rubber dental dam was then applied over the entire back area where clipped, and elastoplast tape was used to wrap the entire midsection of the rabbit to keep the dose in place. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevent excessive movement of the rabbits and prevent them from chewing on the taped area. The harness does, however, allow the rabbits to eat and drink during the dosing period.

All dosing procedures were carried out in a fume hood due to the volatile and potentially dangerous nature of the compounds being tested. Rubber protective gloves were worn at all times by the personnel involved in the dosing procedures. The rabbits were housed in individual cages kept in the hood during the dosing period.

All doses were kept in contact with the rabbit's skin for 24 hours. After this period of time elapsed, the tape, latex, and gauze were removed and the harnesses taken off. The rabbits were kept in individual cages postexposure and observed for death or other toxicity symptoms during the 14 days immediately following exposure. Any deaths which occurred in this period were included in the final tally.

All liquids were applied undiluted where possible. The solid was applied as a powder held in place with gauze patches but not the rubber dental dam.

LD_{50} determinations were calculated using the moving average interpolation method described by Weil (1952). Three rabbits were tested at each dose level. The maximum dose level tested for nontoxic compounds was 20 gm/kg.

Acute Inhalation LC_{50} - Rats

Groups of 10 male rats are being exposed for one hour to a spray, consisting of both vapor and aerosol, of each test material. Concentration levels are varied to achieve partial mortalities necessary for calculation of an LC_{50}. The inhalation LC_{50} determinations will be calculated using the probit analysis method of Finney (1952).

Generation of the paint formulations will be done using a commercial paint sprayer. The spray will be generated into a plexiglas mixing chamber prior to being drawn into the exposure chamber by negative pressure. Regulation of the sprayer and/or the air passing through the mixing chamber will control the concentration as well as the particle size of the aerosol entering the exposure chamber.

Analysis of the chamber concentration will include the vapor concentration of the major solvent as well as the aerosol concentration. A droplet size analysis will be done on the aerosol from a single exposure of each test material.

Pathology Examinations

Gross pathology examination is performed on any animal that dies or is sacrificed following the 14-day observation period. Histopathology is being done on a representative number of rats from each inhalation exposure, both those that die as a result of chemical insult and those sacrificed after the 14-day observation period.

Major organs are taken for histopathology with particular attention directed to the lungs.

Results to Date

Oral dosing of rats to the five paint formulations has been completed with the following results:

Formulation	LD_{50} (95% C.L.) in ml/kg	Data Used to Calculate LD_{50} in ml/kg (Mortality Response, N=5)
134	9.3(6.3-13.8)	5(0), 10(3), 20(5)
1020A	10.0(3.1-32.0)	5(2), 10(2), 20(4)
121/63	16.8(6.7-42.0)	10(1), 20(3)
129/63	--	10(0), 20(2)
105	--	10(0), 20(1)

Less than one-half of the rats died at the highest dose level (20 ml/kg) for two of the five compounds. Both of these formulations could be considered less than toxic by the per-oral route. The survivors of the higher dose levels of formulation 1020A were quite moribund at sacrifice indicating a persistent toxicity with this compound. This formulation is the only one containing tributyltin compounds instead of cuprous oxide as the active ingredient.

All six compounds have been tested for irritation effects on the intact and abraded skin of rabbits. The materials were found to be nonirritating to the intact or abraded rabbit skin when examind at 24 and 72 hours.

Evaluation of the Acute Intraperitoneal Toxicity and the Skin Sensitization Potential of OMP-1 AND OMP-2

A series of organometallic polymers (OMP) have been investigated for use as antifouling agents in paints used on ship bottoms. Dyckman et al. (1973) have described the environmental compatibility of these highly effective agents against barnacles, tubeworms, algae, hydroids, sponges, and bacteria. The polymers containing trialkyltin have been shown to exhibit some degree of mammalian toxicity. Miller et al. (1976) have found poly(tri-n-butyltin methacrylate) (OMP-1) to have an oral LD_{50} in rats of 230 mg/kg and a 4-hour inhalation LC_{50} of 64 mg/m^3. OMP-1 was also irritating to the skin and eyes of rabbits. Poly-(tri-n-butyltin methacrylate/methyl methacrylate) (OMP-2) has an oral LD_{50} in rats of 280 mg/kg and a 4-hour LC_{50} of 51 mg/m^3 (Naval Medical Research Institute letter report, 1976). OMP-2 was also irritating to the skin and eyes of rabbits.

The purpose of this study was to evaluate the skin sensitization potential and the intraperitoneal toxicity of OMP-1 and OMP-2 for comparison with the information previously obtained for OMP-4 and OMP-5 (MacEwen and Vernot, 1978).

Male Sprague-Dawley rats weighing between 200 and 300 grams and male ICR mice were used for the intraperitoneal LD_{50} determinations. Male Hartley derived guinea pigs weighing between 350 and 400 grams were used for the skin sensitization tests.

OMP-1 and OMP-2 were furnished as 50% solids in mineral spirit. The physical properties of OMP-1 are given below:

Molecular weight	-	6000
Specific gravity	-	1.28
% Sn	-	29.4
Standard acid value of polymer	-	36.0

The physical properties of OMP-2 were not available.

Acute Intraperitoneal Toxicity - LD_{50} Determination

The materials were administered as a single injection into the peritoneal cavity of rats and mice. Groups consisted of five animals per dose level. The animals were held for 14 days following administration of the materials, and any animal that died during that time was included in the final mortality calculations. Animals that survived the 14-day observation period were weighed and sacrificed at that time. The LD_{50}'s with the 95% confidence limits were calculated using the moving average interpolation method of Weil (1952).

Skin Sensitization

Groups of test animals consisted of 20 male albino guinea pigs. The materials were topically applied as a mineral spirit preparation. The normal route of administration of test substances for this test is intradermal injection. However, the injection of mineral spirits proved to be extremely irritating and could not be used. The sensitization test was started on a Monday when the guinea pigs were weighed and closely clipped on the scapular areas. A volume of 0.05 ml of a 0.1% dilution of the test material was administered at the upper right scapular area of each guinea pig. A similar control administration of the vehicle was made at the upper left scapular area. Readings were taken 24 hours and 48 hours later and recorded.

Doses of 0.1 ml of the same dilutions (freshly prepared) were then administered at the clipped dorsal lumbo-sacral areas of the guinea pigs on the following Wednesday, Friday, Monday, etc., until seven doses had been administered. The guinea pigs were rested for three weeks (incubation period), weighed, and given a challenge dose of 0.05 ml of the appropriate dilution of the test material at the lower right scapular area. The left scapular area was again used for vehicle control tests. The reactions were read after 24 and 48 hours and recorded.

The grading system was designed so that the intensity of the skin reaction was represented by a proportionate numerical value and also that any reaction elicited by the vehicle was subtracted from the reaction elicited by the test material and vehicle combined.

The product of the width and length of the wheal (in mm) was multiplied by the following reaction scores:

0 = needle puncture ("np") - no wheal
1 = very faint pink ("vfp") - no value for this reaction
2 = faint pink ("fp")
3 = pink ("p")
4 = red ("r")
5 = bright red ("R")
6 = edema - <1 mm in height ("e")
7 = edema - >1 mm in height ("E")
*8 = necrosis - <1 sq. mm ("n")
*9 = necrosis - >1 sq. mm ("N")

*The product of width and length of the necrotic area multiplied by 8 or 9 was added to the numerical value of the foregoing reactions that were present - calculated in the same manner.

A final grade of 25 or less indicated no sensitizing potential, and a final grade of 100 indicated a moderate sensitization potential.

Results

The results of the acute intraperitoneal toxicity of OMP-1 and OMP-2 are listed in Table 26.

TABLE 26. ACUTE INTRAPERITONEAL TOXICITY OF OMP-1 AND OMP-2

Compound	Species	Data Used to Calculate LD_{50} in mg/kg, N = 5 (Number of Deaths)	LD_{50} (95% C.L.) in mg/kg
OMP-1	Rat	0.78(0), 1.56(1), 3.13(3), 6.25(4)	2.9 (1.4-5.7)
OMP-1	Mouse	6.25(0), 12.5(0), 25(4), 50(5)	20.3 (15.0-27.5)
OMP-2	Rat	1.56(0), 3.13(3), 6.25(3), 12.5(3)	4.4 (1.7-11.1)
OMP-2	Mouse	6.25(0), 12.5(3), 25(4), 50(5)	13.4 (8.3-21.8)

Five mice and 10 rats were used as control animals and received an injection of mineral spirit at a volume similar to that used for the OMP administration. No deaths occurred in the mice while one rat died after mineral spirit injection.

These results are similar to those obtained for OMP-4 and OMP-5 in that the rat was slightly more sensitive than the mouse to the intraperitoneal toxicity of the OMP compounds. The rat and mouse intraperitoneal LD_{50} estimations obtained for OMP-1 and OMP-2 are very similar to those obtained for OMP-4.

Neither OMP-1 nor OMP-2 produced any evidence of skin sensitization in guinea pigs after topical application. Areas of very mild irritation were seen which corresponded to the spreading pattern of the topically applied OMP materials. The irritation was also evident at control sites where only the mineral spirit vehicle was applied. It appeared that the mineral spirit vehicle was causing the skin to dry out resulting in some irritated areas.

Evaluation of the Eye and Skin Irritation Potential of JP-5 Jet Fuel Derived from Petroleum and Shale Oil Sources

The THRU is presently involved in a series of inhalation studies to evaluate the toxic and oncogenic potential of the jet fuel JP-5 derived from petroleum as well as shale oil sources. Very little information presently exists on the irritation potentials of the fuels derived from these two sources. It was the purpose of this study to provide that information.

Following are the military specifications (MIL-T-5624K) for JP-5:

Aromatics, vol. % max.	-	25.0
Olefins, vol. % max.	-	5.0
Mercaptan sulfur, wt. % max.	-	0.001
Sulfur, wt. % max.	-	0.40
Flash point	-	60 C
Density (min-max), kg/m^3	-	788-845 (15 C)
Freezing point max.	-	-46 C
Viscosity, max. mm 2/s (centistrokes)	-	8.5 (-20 C)

The JP-5 (petroleum) jet fuel used for these studies was the same as that used in the 90-day inhalation study of JP-5. The JP-5 (shale) jet fuel is derived from hydrotreated Paraho shale oil. The sample of JP-5 (shale) that was used did not contain all of the necessary additives that are required to meet final military specifications.

The methods used for evaluation of eye irritation and primary skin irritation were the same as those described for the testing of antifouling paints earlier in this report.

Neither material, JP-5 (petroleum) or JP-5 (shale), produced any visible signs of irritation or damage to the cornea, iris or conjunctival of the treated rabbit eyes.

Primary skin irritation tests conducted on rabbits resulted in primary irritation scores of 0.04 and 0.33 for JP-5 (petroleum) and JP-5 (shale), respectively. These scores were based on some very mild erythema. The score values are very low, and both materials would be considered nonirritating.

AIR FORCE MATERIALS

Acute Toxicity, Irritation and Sensitization Potential of Tricyclodecane (JP-10)

JP-10 is a synthetic saturated polycyclic hydrocarbon which, because of its high density and other properties, is being utilized as a jet fuel either alone or as a major constituent (70%) of JP-9 fuel. In the latter application, it has been substituted for RJ-4, a reduced dimer of methylcyclopentadiene, which had undergone chronic toxicity tests in 1974. Since no information has appeared in the literature concerning the toxicological properties of JP-10, a series of acute studies were done to characterize it and provide background information.

This experiment was conducted to provide some basic information on the acute toxicity of JP-10 prior to initiation of a one-year chronic inhalation study on the material.

JP-10 is a single chemical entity identified as tricyclo(5.2.1.$0^{2,6}$) decane. Gas chromatographic analysis of samples from two drums of this material indicated that it is 98% pure JP-10 and 2% miscellaneous unidentified impurities. The material is supplied by the Air Force and was received from Suntech, Ic., Marcus Hook, Pennsylvania.

The known physical properties of JP-10 are as follows:

Molecular weight	-	136
Boiling point	-	360 F
Density, 70 F	-	0.940
Viscosity, 70 F	-	3.5
Flash point	-	135 F
Vapor pressure	-	Unknown.

The acute toxicity tests performed, as well as the species tested, are as follows:

1. Oral LD_{50} - male and female Fischer 344 rats
2. Intraperitoneal LD_{50} - male and female Fischer 344 rats
   - female C57Bl/6 mice
   - male Golden Syrian hamsters
3. Inhalation
   a. Two-hour saturated vapor - female Fischer 344 rats
      - female C57Bl/6 mice
      - male Golden Syrian hamsters
   b. Four-hour LC_{50} - male and female Fischer 344 rats
      - female New Zealand albino rabbits
4. Sensitization - male Hartley strain guinea pigs.

Undiluted JP-10 was administered orally to groups of five male and female rats, male hamsters, and female mice. The maximum dosage level was 20 ml/kg of body weight. If no deaths or fewer than 50% occurred at that level, no higher concentration was tested. This dose is based on the maximum volume which the stomach of the animals can contain without mechanical injury.

Glass syringes with special dosing needles were used to administer the material to the animals. The animals were fasted for at least 16 hours prior to oral dosing. This allowed for more uniform absorption since the amount of food in the stomach varies greatly from animal to animal in the unfasted state. The animals were weighed individually at the time of dosage to determing the proper dose volume.

If the data permitted (i.e., sufficient mortality occurred), the acute oral LD_{50} and its 95% confidence limits was calculated using the moving average interpoltion method of Weil (1952). Any death which occurred during the 14-day observation period following the administration of the single dose was included in the final mortality tally. Any animal that survived the 14-day postdosage period was weighed and sacrificed at that time.

A single dose oral LD_{50} for JP-10 could not be determined in either rats or hamsters using the standard oral dosing techniques. Doses administered below 10 ml/kg failed to produce deaths in either species (Table 27). The oral LD_{50} for both species is greater than 20 ml/kg and would not be considered toxic by accepted toxicity standards. Dosing at concentrations higher than 20 ml/kg involves large volumes which could cause mechanical injury to the animal's stomach.

TABLE 27. ACUTE ORAL TOXICITY OF TRICYCLODECANE (JP-10) IN RATS, HAMSTERS, AND MICE

Dose (ml/kg)	Male Rats	Female Rats	Mice	Hamsters
20.00	1/5	2/5	5/5	2/5
10.00	2/5	1/5	4/5	1/5
5.00	0/5	0/5	4/5	0/5
2.50	0/5	0/5	1/5	0/5
1.25	-	-	0/5	-
LD_{50}, ml/kg	-	-	3.9	-
95% C.L., ml/kg			2.2-6.9	

Mice proved to be less resistant to the toxic effects of JP-10 administered by this route with a resultant LD_{50} of 3.9 ml/kg. Deaths occurred within 48 hours of dosing with convulsions immediately preceding death.

Groups of five male and female rats, female mice, and male hamsters received single intraperitoneal (IP) injections of JP-10. Doses were administered to each group on a ml JP-10/kg body weight basis at varying levels for the purpose of calculating an LD_{50}. The acute intraperitoneal LD_{50} and its 95% confidence limit was calculated for each species using the moving average interpolation method of Weil (1952). Any death which occurred during the 14-day observation period following the administration of the dose was included in the final mortality tally. Any animal that survived the 14-day observation period was sacrificed at that time.

Single injection IP LD_{50}'s were determined in all species (Table 28). Although mice still appear to be the most susceptible species, the differences between the different species are slight.

TABLE 28. ACUTE INTRAPERITONEAL TOXICITY OF TRICYCLODECANE (JP-10) IN RATS, HAMSTERS, AND MICE

Dose (ml/kg)	Male Rats	Female Rats	Mice	Hamsters
4.0	5/5	5/5	5/5	5/5
2.0	4/5	4/5	5/5	4/5
1.0	2/5	0/5	2/5	1/5
LD_{50}, ml/kg	1.2	1.6	1.1	1.4
95% C.L., ml/kg	0.8-2.0	1.2-2.2	0.7-1.6	0.8-2.5

The following patch-test method was used to measure the degree of primary skin irritation of intact and abraded skin in albino rabbits:

All six rabbits tested were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Two areas on the back, one on each side, were designated as patch areas. One area was abraded by making minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. This was made in a square pattern with a syringe needle used to make the incisions.

The JP-10 was applied in the amount of 0.5 ml per site. The material, applied to the designated patch areas, was covered by a 1-inch square or surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was then covered with a rubber dental dam strip and secured with more elastoplast tape. These patches remained in place on the rabbits for 24 hours. During that time, the rabbits were fitted with leather restraining collars to prevent disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water during the test period.

After 24 hours, the wrap and patches were carefully removed, and the test areas evaluated for irritation using the Draize table as a reference standard. Readings were made at 72 hours also (48 hours after the first reading).

One-tenth milliliter of the undiluted JP-10 was applied to one eye of each of six albino rabbits. The opposite eye remained untreated and served as a control. Examinations for gross signs of eye irritation were made at 24, 48, and 72 hours following application. Scoring of irritative, iris, and conjunctival effects are scored separately. In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score. Cornea and iris scores are heavily weighed because of the essential role of these organs in vision.

The results of both of these irritation tests were negative. JP-10 caused no irritation to the eyes or to either abraded or intact skin after 24, 48, or 72 hours.

Twenty guinea pigs were given seven sensitizing intradermal injections of JP-10 on a Monday, Wednesday, Friday basis. Following a three-week incubation period, the guinea pigs received a challenge injection. Reactions were graded at 24 and 48 hours postinjection.

Eight of the twenty guinea pigs showed a sensitization response with a mean score of 43. The number responding and the mean score indicates that JP-10 has a moderate potential for sensitization and produces a mild response.

Groups consisting of six male and female Fischer 344 rats, six male Golden Syrian hamsters, and six female C57Bl/6 mice were exposed to saturated vapors of JP-10 for two hours. The saturated vapor generation was achieved by passing air through a bubbler tower at a flow rate of 2.0 liters/minute. The effluent air was passed into a 9-liter inhalation chamber. The concentration of JP-10 in the air was estimated by weighing the JP-10 containing bubbler before and after the exposure. The experimental animals were observed for toxic signs at frequent intervals during the exposure period and at least once daily thereafter for 14 days. The animals were weighed just before exposure and at days 1, 3, 7, and 14 postexposure. At the end of the 14-day observation period, the animals were sacrificed and a gross necropsy performed. Any animals that died during the observation period were subjected to gross necropsy. If saturated vapor exposures resulted in significant animal mortality, 4-hour LC_{50} values were determined. In this event, the concentrations of JP-10 in the chamber were determined analytically.

The groups of animals were exposed to a mean concentration of 8.3 mg/ℓ JP-10 during the two-hour exposure. This was approximately 1500 ppm, representative of saturated vapor conditions at room pressure and temperature. Deaths occurred in each animal group except hamsters. All female rats and mice died during or immediately following the two-hour exposure. Five of the six male rats died. Three of these died during exposure. The other two were sacrificed due to hind quarter paralysis and cannibalism by their cage mates. Gross signs of toxicity during exposure included tremors and ataxia. Death during exposure was preceded by clonic convulsions.

Since no deaths occurred in the hamster group during this two-hour saturated vapor exposure, a second group was exposed under similar conditions for six hours. Again, all hamsters survived.

The results of the 4-hour LC_{50}'s to rats and mice are shown in Table 29. Essentially no difference in toxicity was noted between the sexes of rats as each showed an LC_{50} of approximately 1200 ppm. High level exposures again produced hind quarter paralysis in both sexes of rats.

TABLE 29. FOUR-HOUR INHALATION TOXICITY OF TRICYCLODECANE (JP-10) IN RATS AND MICE

Male Rats		Female Rats		Mice	
Conc. (ppm)	Mortality	Conc. (ppm)	Mortality	Conc. (ppm)	Mortality
1318	6/6	1440	6/6	955	6/6
1260	5/6	1240	4/6	900	0/6
1224	2/6	1151	2/6		
1157	1/6	728	0/6		
LC_{50}, (ppm)	1221		1194		930 (ALC_{50})*
95% C.L. (ppm)	1174-1259		1107-1287		

*Approximate LC_{50}; not enough partial mortality data to calculate an LC_{50}.

In the mouse exposures, the concentration range between 0 and 100% mortality was so small that attempts to achieve partial mortality would not have been worthwhile. Although the LC_{50} is approximate due to the lack of partial mortality, it is probably quite accurate because of the narrow concentration range between zero and total mortality. Exposure of hamsters to a saturated vapor of JP-10 for 6 hours failed to cause any mortality during exposure or the subsequent 14-day observation period.

Gross pathology findings in the animals that died were limited to multifocal congestion and areas of emphysema scattered throughout the lungs. The livers were occasionally swollen with a reticulated appearance.

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall program of the laboratory.

ENVIRONMENTAL HEALTH AND SAFETY PROGRAMS

At the time of protocol preparation during the planning for inhalation exposures, consideration is given to the possible hazard to laboratory workers caused by escape of the volatile contaminant. As reported in the last annual report (MacEwen and Vernot, 1978), a system was designed for sampling the atmosphere in each of the exposure areas of the THRU. This system has sampling ports at points in the laboratory where leakage of contaminant is considered to be most probable. The air is sampled sequentially at each of these ports by means of a pump-timer-solenoid combination which delivers the sample to an analytical instrument chosen for its sensitivity and/or specificity.

This system has been used during the last year to check levels of contamination by the hydrocarbons being introduced into the large (840 cubic feet) exposure chambers. The Beckman Model 400 Hydrocarbon Analyzer was selected as the analytical instrument because of its high sensitivity. Two analyzers were used, one in Laboratory A and the other in Laboratory B.

Each analyzer samples five points and an outside air baseline twice each hour. The sample lines are stainless steel which was found least reactive for hydrocarbon samples. When not sampling, the air is continuously pulled through the sample lines at 2 liters/minute using an auxiliary pump. Each line is switched on and off using a Skinner electric two-way valve. The sample is pumped into the hydrocarbon analyzer with a single stage Diapump. The Beckman Model 400 Hydrocarbon Analyzers were calibrated with propane in air

standard bags. The hydrocarbon analyzer signal is recorded on a Heath SR255B millivolt recorder and the output is monitored by an adjustable alarm system constructed by the THRU Facility Engineering Department. The audio-visual alarm system is set to notify personnel when hydrocarbons have exceeded 25 mg/m^3, equivalent to 5 ppm of gasoline.

The hydrocarbon analysis is a very sensitive measure of atmospheric hydrocarbon concentration but does not provide identification. For this reason, a gas chromatographic identification method was developed to tentatively determine if the hydrocarbon analyzer response is due to one of our contaminants and in what concentration.

The Varian 1200 with a 1/8" x 12' stainless steel 10% SE 30 on 80/100 mesh chromosorb WAW column is being used isothermally at 110 C. The contaminants of current interest are MCH, JP-10 and JP-5, and decalin was a chamber test material early in this report period. The JP-5 is a mixture so it is not as easily identified as decalin, MCH or JP-10, but toluene, which is one of the most abundant volatiles in the mixture, is being used as an indicator of JP-5 contamination. Quantitation is done on the basis of absolute peak area as calculated by the Spectra-Physics System I computing integrator. Instrument calibrations for JP-10 and MCH were done by conventional methods.

The hydrocarbon industrial hygiene analysis was started in June of 1978. There have been twelve excursions in excess of 25 mg/m^3 due to our contaminants through 23 April 1979 as listed in Table 30.

TABLE 30. CONTAMINANT EXCURSIONS IN EXCESS OF 25 mg/m^3 IN THE LARGE CHAMBER LABORATORY

Date	Time	Sample Point	Cause
6/9/78	Undetermined	Chamber 7 Generation Hood	Decalin
6/22/78	1600	Chemistry Laboratory	Decalin
8/9/78	1300	Chamber 7 Generation Hood	MCH
8/15/78	1430	Contaminant Generation Area	MCH
8/17/78	1430	Contaminant Generation Area	MCH
8/21/78	1300	Contaminant Generation Area	MCH
8/21/78	1500	Contaminant Generation Area	MCH
10/5/78	0800	Contaminant Generation Area and Chamber Area	MCH
10/6/78	0800	Contaminant Generation Area	MCH
10/6/78	1500	Contaminant Generation Area	MCH
10/17/78	1030	Contaminant Generation Area	MCH
10/17/78	1130	Contaminant Generation Area	MCH

The frequent excursions of MCH noted in October led to an examination of the system to determine the source of the leakage. It was found that there were two points of contamination of laboratory air: (1) a leak in the tubing connection at the contaminant supply drum, and (2) the fact that the exhaust from the large chamber CO_2 analyzer system was being exhausted into the laboratory. The first problem was eliminated by tightening tubing connections and by constructing an exhaust hood over the supply drum. The second was taken care of by shutting down the CO_2 analyzer system which was no longer necessary with the chambers operating under ambient atmosphere and pressure conditions.

There were 75 other excursions in excess of 25 mg/m^3. Forty-two were due to painting, 18 were caused by cleaning solvents, 3 resulted from floor sealant, 2 were caused by the use of gasoline in the building by construction contractor personnel, 2 from acetone used in the chemistry laboratory, 1 from butane in the chemistry laboratory, 1 from welding flux, and the final 6 were of unknown causes. The unknowns were of very short duration, 1-10 minutes, and all on off shifts.

Laboratory air concentration in the small chamber laboratory is being monitored for UDMH contamination during the periods of mouse exposure to 5 ppm purified UDMH. An MDA Model 7020 hydrazine monitor is being used at its most sensitive limit which is 2 ppb. An audio-visual alarm system is activated when the concentration rises to 50 ppb, which is one-tenth the present TLV. Each new tape is recalibrated at 50 ppb and the alarm point readjusted, if necessary. The atmosphere sampling points are immediately above and in front of the contaminant introduction hood. During the year, there were 3 excursions over 50 ppb. These occurred on 11/6/78, 3/2/79, and 3/15/79. They were all caused by temporary power failures during which the contaminant either diffused or was pulled out of the introduction hood. During these excursions, the laboratory was evacuated until levels of UDMH dropped below 50 ppb. Efforts are now being made to provide the ambient laboratory with emergency power so that exposures can continue during power outages without contamination of laboratory air.

During this report period, Mr. M. Schneider was given responsibility for environmental health and safety programs at the THRU. Major programs which were implemented under his guidance included institution of smoking, eating and drinking prohibitions in all THRU laboratory areas and implementation of a policy governing safety eyeglass requirements in all laboratory areas. All personnel with responsibility in laboratory areas were fitted with safety glasses, including prescription lenses, and required to wear them in laboratory areas at all times.

COMPUTER PROGRAM DEVELOPMENT

The capability of the ASD computer to accept data for inclusion in a memory data base and to permit retrieval of that data under a variable system of groupings was utilized in setting up programs for input and retrieval of (1) analyzed chamber concentration data measured during inhalation experiments and (2) mortality data for all animals in a study.

The programs utilize VENUS, a language designed for simple interactive communication between user and computer. The major advantage of VENUS is that input by the user follows prompting by the computer so that errors can be detected and identified by the computer at the time of occurrence. The VENUS programs can be easily learned by people inexperienced in computer programming or operation and have been transferred to the users in the Chemistry and Laboratory Operations Departments.

The chemistry data programs developed are titled CHEM for the data storage program and MEAN for the data retrieval program. Program CHEM permits insertion of experiment number, contaminant and concentration, date, and responsible chemist's name. Enough points selected from the graph of the output of the contaminant analyzer to provide a satisfactory mean value are inserted daily into the computer. A conversion factor calculated from periodic calibration and a baseline constant measured on the day of analysis are typed into the keyboard. The internal computer program then computes the concentration at each data point and files it in memory for future retrieval. When inserting concentration data, a specific code may be selected which will store the data as ppm, ppb or mg/m^3.

Using program MEAN, four options are available for retrieval of data, each called up by a different code. One returns all of the analytical data inserted into the computer memory for any experiment; one yields all analytical data obtained on a particular chamber; one provides individual chamber data prior to a desired date either as daily means, standard deviations and ranges or as a mean for the period with standard deviation and high and low days; the last option gives the same data but over the whole length of the study.

Program PATH is the entry program for mortality data. Animals are identified by experiment number, species and animal number. The date of death is typed in as a Gregorian calendar date and converted in the computer to number of elapsed days from the start of the study. Codes are available for characterizing death status of the animal and include: lost or missing, cannibalized, autolyzed, natural death, moribund and scheduled sacrifice.

If examination of pathology sheets demonstrates that a record has been entered which contains erroneous information, procedures are available in the program for deletion and replacement of records. Deletion is performed when it is found that an animal listed as dead is still alive. A record which is found to contain an erroneous death status or date of death is replaced by one with the correct information.

Two programs, STAT and MORT, are available for retrieval of mortality data. In program STAT, current mortality is provided by the computer in one of three ways: (1) for all animal species in a group of specified experiments; (2) for all animal species in a particular experiment, and (3) for one selected species in a particular experiment. In order to broaden the utility of this program, efforts are now underway to include the pathology accession number as animal identification and to permit retrieval of mortality information as of any selected date within the experiment. This will make the program useful to the Air Force Pathologists since animals are identified in the Pathology Branch (THP) by means of accession numbers.

Program MORT was designed to aid in comparison of mortality rates, either between exposed and control animals or among different experiments. The percent cumulative mortality is printed for each 30-day period having elapsed since the start of the exposure. In calculation of percent mortality, missing animals and those sacrificed on schedule are not included in either numerator (dead animals) or denominator (total animals) since these do not reflect any exposure effect.

In addition to the foregoing programming development, the Mathematics Department has continued its project of upgrading old programs and changing programs to reflect changes in ASD computer hardware or software.

GAS CHROMATOGRAPH-MASS SPECTROMETER-DATA SYSTEM (GC-MS-DS)

The Chemistry Department's analytical capabilities have greatly been increased by recently acquiring a Hewlett-Packard 5993A Gas Chromatograph-Mass Spectrometer. The GC-MS-DS was received in January 1979 and set up by the Hewlett-Packard service engineer in February 1979. The data system includes a HP1000 E-series computer with 32k of semiconductor memory, a fixed and a removable disc system HP7900A dual-disc drive, a HP2648A graphics terminal with viewscreen and keyboard, and a Tektronix 4632 video hard copy unit. The powerful combination of hardware is supported with an excellent software package. The fixed disc contains the software control programs which run, tune, and troubleshoot the instruments. It also contains programs for data collection and analysis. The analysis programs include visual spectra and GC comparison and a computer matching library included spectra. At present, the GC/MS is being used to quantitatively measure and detect specific toxic compunds at very low concentrations, trace impurities in contaminants and for identification of components in jet fuels. A capillary interface system will soon be installed for additional capability of identification of metabolites from biological or physiological fluid samples. A contributed library and a NIH/EPA spectral library will be added to the system. These libraries contain spectra from standards run on all types of instrumentation. The fits may not be as good as standards run on our instrument but will greatly broaden our identification capabilities.

VAPOR PRESSURE OF JP-9 JET FUEL CONSTITUENTS

There was some uncertainty concerning the contributions of the individual components of JP-9 to the equilibrium vapor pressure of the mixture. JP-9 is a mixture of 67% by weight of JP-10, 22% RJ-5 (a mixture of reduced dimers of bicycloheptadiene) and 11% methylcyclohexane. Since the inhalation toxicity of the vapor of the mixture is a function of the toxicities of the individual components and their relative concentrations in the vapor, it is important to know what these concentrations are. Therefore, a synthetic JP-9 mixture was made up from the individual components and a liquid sample analyzed gas chromatographically to give the chromatogram shown in Figure 20. As can be seen, each of the components is not a completely pure compound with RJ-5 being a mixture of 3 isomers in the proportion of 75:15:10. When the areas under the peaks were measured using a computing integrator, the following weight percentages of the components were calculated: MCH - 11.0%, JP-10 - 67.3%, and RJ-5 - 21.0%. This confirmed that the synthetic mixture had been formulated properly.

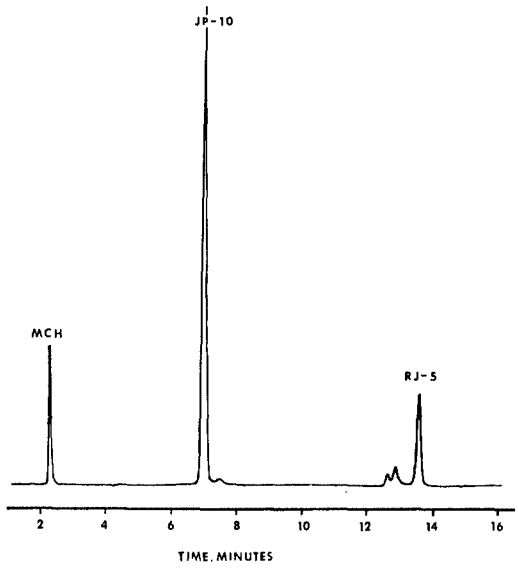


Figure 20. Gas chromatogram of a synthetic JP-9 mixture.

After equilibration at 74 F, the headspace over the synthetic JP-9 was sampled and analyzed to give the gas chromatogram shown in Figure 21. The same peak distribution was obtained after 2 and 24 hour periods of standing indicating that vapor equilibration had been achieved. Calculation of the areas of the peaks gave the concentrations of the individual components shown in Table 31.

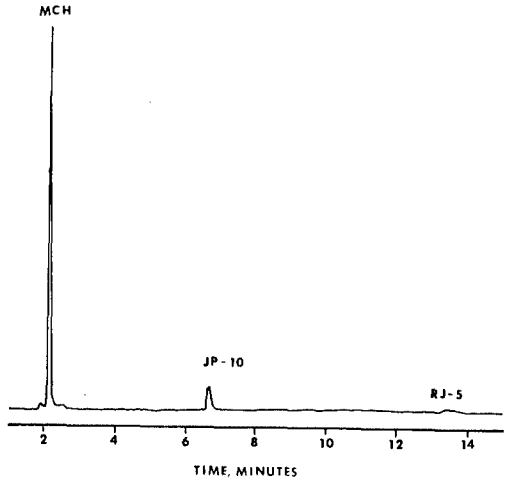


Figure 21. Gas chromatogram of headspace over synthetic JP-9 mixture.

TABLE 31. CONCENTRATIONS OF JP-9 CONSTITUENTS IN SATURATED HEADSPACE

Component	Concentration		
	ppm	Torr	Relative, %
MCH	9400	7.15	92.0
JP-10	660	0.50	6.5
RJ-5	160	0.12	1.5

The data demonstrate that MCH contributes the greatest vapor concentration even though its liquid concentration is only 11%. If the MCH concentration in a vapor mixture is controlled to 400 ppm, the present TLV for this substance, the resulting JP-10 concentration would be about 30 ppm and that of RJ-5 about 6 ppm.

PHYSIOLOGICAL FLUIDS - DETERMINATION OF GAS CHROMATOGRAPHIC PEAK CONSTITUENTS

The gas chromatographic (GC) analysis of physiological fluids to examine effects of intoxication was originally developed using packed columns of Tenax, an uncoated porous polymeric material. With the acquisition of the Varian 3700 gas chromatograph last year, it became possible to perform capillary GC which is superior to conventional packed GC in resolution, retention time, and column reactivity. Therefore, the capillary column accessory for the Varian 3700 GC was ordered and installed and work begun to develop a method for urinary fingerprint and metabolite analysis.

Since a year-long intermittent exposure to methylcyclohexane had begun and rat urine could be collected easily during the nonexposure hours at night, it was decided to investigate the excretion of methylcyclohexane metabolites in rat urine. The major metabolites of methylcyclohexane can be expected to be limited to the isomeric methylcyclohexanols and their conjugates and the methylcyclohexanones. It was possible to obtain from commercial sources the 3 methylcyclohexanol isomers and 4-methylcyclohexanone.

Capillary columns, 50 meters in length, coated with different phases, were tested for applicability to separation of volatiles in urine, including FFAP (free fatty acid phase) OV 101 (a methyl silicone polymer) and Carbowax 20M (polyglycol phase). The FFAP gave very poorly shaped peaks.

OV 101 gave well separated peaks but some were not as sharp as would be expected from capillary work. The Carbowax 20M column yielded very sharp, well separated peaks throughout the program. Carbowax 20M had more column bleed than OV 101 but after several weeks of conditioning, the baseline rise did not interfere. Coating loss was the cause of the column bleed, and there was a resultant small continuous decrease in retention times. For these reasons, the 0.25 mm ID X 50M Carbowax 20M was used with a 0.5 ml/minute helium flow and oven temperature program of 32 C for 10 minutes, 4 C per minute to 200 C.

Initially, it was thought that solvent extraction of the untreated urine, or after acid hydrolysis, would provide a satisfactory sample for GC analysis. Invariably, extraction, whether with solvents heavier or lighter than urine, gave emulsions which were difficult to break and/or precipitates which were difficult to separate from the organic liquid phase. The solvents, even though of chromatographic grade, all contained impurities which interfered with interpretation of the chromatograms. As a result of the problems associated with solvent extraction, direct injection of urine into the GC was investigated. The sample was injected into an insert filled with glass beads to retain the solids in the urine. The splitter was set to allow 10% of the injected volatiles into the Carbowax 20M column. This method did not lead to plugging of the capillary column and provided peaks of reasonable intensity. The Spectra Physics Model I computing integrator was used to process the data. The program was set up specifically to process a capillary chromatogram with sharp narrow peaks and quick return to baseline.

The method of urine collection in metabolism cages was altered somewhat to eliminate urine loss during collection by clamping the collection funnel in place to prevent movement. With the exact amount excreted known, the peak areas could be normalized to eliminate, as far as possible, the differences in urine concentration among rats. Normalization is accomplished by calculating the average urine volume excreted nightly by the rats being sampled and adjusting the peak area in the following manner:

$$\frac{\text{Volume Sampled}}{\text{Average Volume Excreted}} \times \text{Peak Area} = \text{Normalized Area.}$$

Examination of GC data obtained before and after normalization indicated that there was a significant reduction in the range of values for 25 peaks, no difference for 5 peaks and a significant increase in the range for 3 peaks. Urine samples are routinely collected for chromatography at 0900 hours.

The compounds obtained as possible methylcyclohexane metabolites were run under the conditions developed for urine analysis. Solutions of 50 ppm were made in water and urine and chromatogrammed with another injection of the urine sample made after 2 hours standing to note any changes. Each methylcyclohexanol gave 2 major peaks, probably reflecting the presence of cis-trans isomers in each sample. The peaks of the presumed metabolites eluted in a portion of the chromatogram which was almost free of urine volatile peaks permitting their detection in urine. Table 32 is a summary of the data obtained.

TABLE 32. RETENTION TIMES AND PEAK AREAS OF ISOMERIC METHYLCYCLOHEXANOL AND 4-METHYLCYCLOHEXANONE IN WATER AND URINE

		Peak Areas[1,2]					
		Water		Urine		Urine + 2 Hours	
Compound	Retention Time, Minutes[3]	#1	#2	#1	#2	#1	#2
2-Methylcyclohexanol	40.1	692	649	862	934	474	600
	40.6	2263	2126	2764	2948	1569[4]	2054
3-Methylcyclohexanol	41.2	1129	1060	832	687	736	723
	42.0	2598	2341	1848	1593	1934	1963
4-Methylcyclohexanol	41.3	860	1405	1008	1174	961	975
	22.1	1472	2312	1678	1989	1717	1675
4-Methylcyclohexanone	37.4	2556	2774	2622	2233	1971	1991

[1]In arbitrary integrator units.
[2]Duplicate injections.
[3]Retention time changes slowly as column bleeds.
[4]3.5 hours.

Reproducibility between duplicates appears satisfactory, and the peaks are the same whether urine or water is used as the medium. As the column is used at higher temperatures, liquid phase slowly bleeds off leading to slowly decreasing retention times. However, since this happens to all the peaks in the chromatogram, it does not present great difficulty in peak identification.

Urine samples were then collected from control rats and animals exposed to 400 ppm methylcyclohexane. Figures 22 and 23 illustrate the chromatograms obtained from control and exposed rat urine, respectively, while Figure 24 is one obtained from a mixture of the known presumed metabolites, 2,3 and 4-methylcyclohexanols and 4-methylcyclohexanone. The most significant difference between the controls and exposed rats is the appearance of 12 peaks at 11.40, 34.20, 35.07, 35.75, 38.70, 38.80, 39.26, 39.77, 40.60, 41.27, 44.46, and 46.50 minutes. The peaks from 38.70 through 41.27 minutes correspond with the peaks shown by the methylcyclohexanols while the 35.75 peak corresponds to 4-methylcyclohexanone. The peaks at 34.20 and 35.07 minutes may be due to the 2 and 3-isomers of methylcyclohexanone although we have not yet run known compounds to make that assignment.

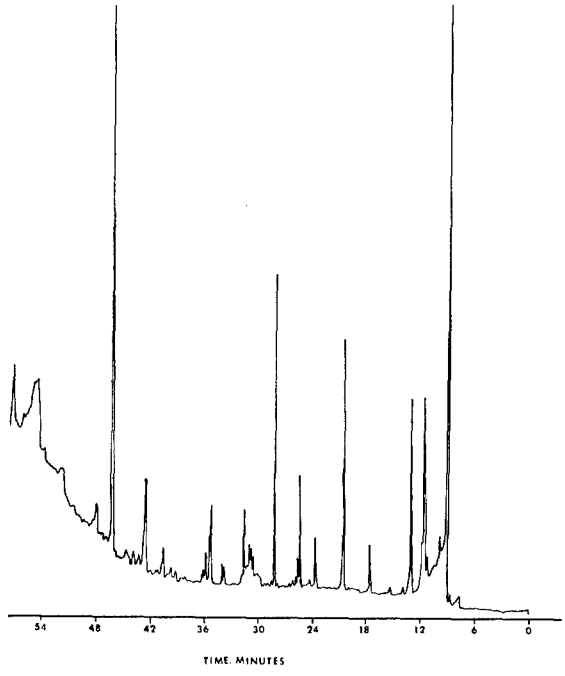


Figure 22. Chromatogram of unexposed control rat urine.

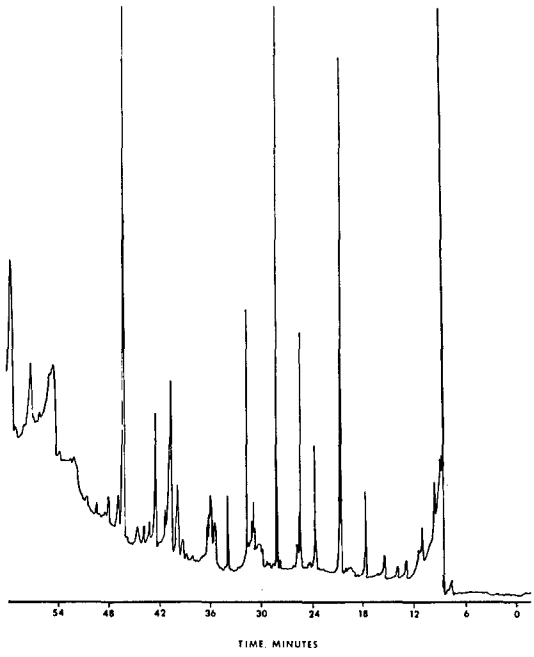


Figure 23. Chromatogram of methylcyclohexane exposed rat urine.

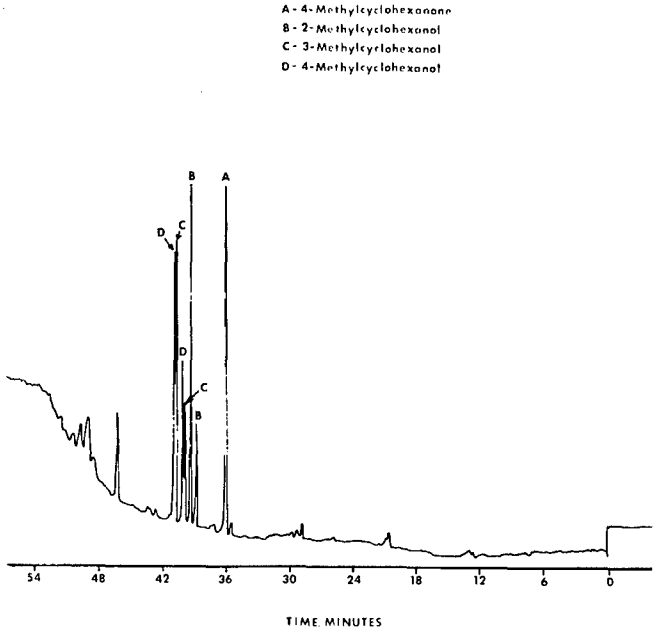


Figure 24. Chromatogram of a mixture of known or presumed rat metabolites of inhaled methylcyclohexane.

TRAINING PROGRAMS

CHAMBER TECHNICIAN

Formal training programs which involve the operation of the inhalation chambers were successfully completed by three chamber technicians hired since the last annual report. The training program was slightly altered in format during the year. An outline of the program is shown below.

I. Orientation
   - A. Laboratory Mission
   - B. Job Responsibilities
     1. General Coverage of SOP's
     2. General Coverage of Laboratory Operations
   - C. Personnel Responsibilities

II. Standard Operation of Chambers
   - A. Observer A - Normal Routine
     1. Dome Start-up
     2. Establish Flow
     3. Normal Readings
     4. Dome Entry Operation
   - B. Observer B - Normal Routine
     1. Dome Entry Operation
     2. Observer B Check List
   - C. Dome Entrant Duties
     1. Dome Entry Operation
     2. Dome Cleaning and Cage Changes
   - D. Dome Cap Raising and Lowering

III. Mechanical Equipment
   - A. Vacuum Pump Failure
     1. Facility A Pump
     2. Facility B Pump
     3. Observer Duties
   - B. Air Compressor Failure
     1. Main Air Compressor
     2. Back-up Air Compressor
     3. Air Dryers
     4. Observer Duties
   - C. Complete Power Failure
     1. Facility A Procedures
     2. Facility B Procedures
     3. Observer Duties
   - D. Air Supply Fan Failure
     1. Main Supply Fan
     2. Back-up Supply Fan
     3. Observer Duties
   - E. Waste Catch Tank Draining
     1. Transfer Dome to Tank
     2. Emptying of Tank

IV. Emergencies
- A. Fire in Dome During Entry
  1. Observer A Duties and Options
  2. Observer B Duties and Options
  3. Dome Entrant Duties
- B. Fire in Dome - No Entrant
  1. Observer A Duties and Options
  2. Observer B Duties and Options
- C. Fire in Airlock During Entry
  1. Observer A Duties and Options
  2. Observer B Duties and Options
  3. Dome Entrant Duties
- D. Fire in Exposure Laboratory
  1. Observer A Duties and Options
  2. Observer B Duties and Options
- E. Rescue of Incapacitated Dome Entrant
  1. Rescue Criteria
  2. Observer A Duties
  3. Observer B Duties
- F. Operation of Scott Air Pak (SCBA)
  1. Criteria for Use
  2. Procedures

Each of the above areas involves a combination of classroom training and on-the-job observation. Demonstrations by chamber technicians and facility engineering personnel along with participation by the trainee take place for many of the procedures. Written and/or practical examinations covering all of the outlined topics are given at appropriate times during the training period.

The new technicians were also training in blood sampling, handling, and restraining of all animal species used in the laboratory. Their training also extended to auxiliary equipment such as the electronic animal weighing system.

One of the technicians that was hired was a former chamber technician previously certified by AALAS as an assistant animal technician. The other two technicians completed the Ralston Purina Animal Care self study course and have either job experience or vocational school training which qualifies them for application for certification as an assistant animal technician. Since the last annual report, two technicians were certified as assistant animal technicians, two as laboratory animal technicians, and one as a laboratory animal technologist. The technologist level is the highest level of certification in the AALAS program.

Simulated and deliberate emergency training procedures were conducted by the chamber technicians on a monthly basis during the year. The situations serve as refresher training for all technicians involved. The list of various training procedures is shown below.

Date	Procedure	Personnel Participation
June 1978	Vacuum Pump Failure	All
July 1978	Air Supply Fan Failure	A,B
August 1978	Fire in Dome During Entry	A,B
September 1978	Air Compressor Failure	All
October 1978	Fire in Dome - No Entrant	A
November 1978	Operation of Scott Air Pak	All
December 1978	Fire in Dome With Entrant	A,B,C
January 1979	Fire in Dome - No Entrant	A
February 1979	Rescue of Incapacitated Dome Entrant	A,B,C
March 1979	Fire in Dome During Entry	A,B,c
April 1979	Vacuum Pump Failure	A

A = Observer A
B = Observer B
C = Dome Entrant
All = All Chamber Technicians

The videotapes of the animal care course conducted by the School of Aerospace Medicine at Brooks Air Force Base were transferred to cassettes for viewing by the technicians who had not previously seen these tapes. The course, outlined in a previous annual report (MacEwen and Vernot, 1975), is a valuable training aid and is used by the technicians in preparing for AALAS certification. Four chamber technicians were involved in the viewing of the taped series of lectures.

ANIMAL TECHNICIANS

Since last year's annual report, several animal technicians have become certified in the AALAS program. Two became certified at the first level, assistant animal technician. Applications for AALAS certification have been made by three other technicians at the first level. UCI animal care personnel certification in the AALAS program is as follows:

1. Assistant Animal Technician
2. Laboratory Animal Technician
3. Laboratory Animal Technologist.

Upon satisfying the requirements for and successfully passing the first level AALAS examination, the two above mentioned animal caretakers were promoted to the position of animal technician.

Three animal caretakers have been hired in the past year, and each has successfully completed the Purina Animal Care Course. This course, primarily a self-study course, lays a foundation for further study in the field of laboratory animal care. The caretakers completed the course at their own pace under the direction of a supervisor. Tests covering the four main divisions of the course were taken by the technicians, and all passed with scores greater than 70%.

Animal Care Training Videotapes

The videotapes of the animal care course discussed under chamber technician training were also used in the training of animal care personnel. The training tapes were presented during the work day over a period of one month. The training was organized in a logical sequence and periodic quizzes were given to stimulate interest and learning. A supervisor was present while the taped program was presented to allow for discussion or questions following each session.

Advanced Practical Training

These exercises, as described in detail in a previous report (MacEwen and Vernot, 1977), were made available to the technician group. Technicians are assigned to assist the Research Support section when assistance is requested. This program is continuous as time permits and has added versatility to our animal technician group.

Laboratory Animal Medicine and Audiotutorial Series

This series, also described in detail in a previous report (MacEwen and Vernot, 1978), was made available by the Air Force Veterinary Medicine Division. Approximately one third of this series has been covered by the UCI animal technician group. Each subject within the series is directed by an expert in that particular field and includes audiotapes as well as slides. Discussions and questions are encouraged following each training film.

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